Vol. 95, No. 2 October, 1948

THE
BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

ADDRESSES AT THE LILLIE MEMORIAL MEETING WOODS HOLE, AUGUST 11, 1948

B. H. WILLIER, R. G. HARRISON, H. B. BIGELOW, E. G. CONKLIN

FRANK RATTRAY LILLIE

1870–1947

The Work and Accomplishments of Frank R. Lillie at Chicago

It is my privilege and honor tonight to review and appraise the work and accomplishments of Frank Rattray Lillie at the University of Chicago. The year following his graduation in 1891 from the University of Toronto he was fellow in zoology at Clark University, where he began graduate studies under the direction of C. O. Whitman, one of the early leaders in American zoology and the first director and one of the guiding spirits in the early days of the Marine Biological Laboratory. In 1892 the dynamic President Harper lured Whitman, among other prominent scientists, away from Clark University to the newly founded University of Chicago. With affectionate admiration and devotion every student went with Whitman. There two years later (in 1894) Lillie received, at the age of 24, the degree Doctor of Philosophy in Zoölogy. In accord with the trend in embryology of those days, his thesis dealt with the phenomena of cell lineage as it occurs not in a marine species, but in the fresh water clam, *Unio*. Worthy of special mention here is an evaluation of this work in his own words, written only four years ago: "A feature of special interest in the first publication was a discovery I made in studying the cell-lineage of *Unio*, that the behavior of the individual cells was adaptive and that varying sizes, rates and direction of division of cells were directly related to the subsequent events." To him development at any particular time and place is always a special feature directly related to functional need. Herein lies the key to his philosophy of embryology and of biology in general.

Except for a period of six years following the doctorate, his professional life was intimately associated with the University of Chicago from the time of its founding. During this period he was for 5 years (1894–99) instructor of zoölogy at the University of Michigan and for one year (1899–1900) Professor of Biology at Vassar College. In the fall of 1900 he was called back to Chicago as Assistant Professor of Zoölogy, which in 1906 culminated in a professorship of Embryology at the age of thirty-six years. Upon the death of Whitman in 1910, Lillie succeeded him as chairman of the Department of Zoölogy and continued in that position until 1931, a span of 21 years. From 1931 to 1935 he was Dean of the Division of the Biological

Sciences. Concurrently in recognition of his outstanding achievement in research and service to the university and to the science of biology at large he was the Andrew MacLeish Distinguished Service Professor of Embryology, and thereafter held this title emeritus until his death.

Outstanding among his accomplishments as chairman was an effective departmental organization which was characterized by a lack of elaborate administrative machinery and by a policy which encouraged both freedom and initiative for the individual, whether staff or student. In dignity and simplicity he administered without seeming to do so. His was a leadership in which everyone knew his position, responsibility, and opportunity. With the growth of the department, the need for additional research facilities became increasingly great. This need was met to a large extent in 1936 when he and Mrs. Lillie very generously presented to the university The Whitman Laboratory of Experimental Zoölogy. Named in honor of Professor Whitman, the first head of the department, it stands today as a monument to the research ideals of both teacher and student.

The four-year term as Dean of the Division of Biological Sciences began only one year after the clinical departments of a new medical school were set up on the Midway campus. In Dr. Lillie's own words his "special task as dean was to amalgamate the old established pre-clinical departments with the newly established clinical departments and hospitals into a coherent medical school." Through much patience, vision, and understanding he succeeded in uniting the departments of basic sciences and clinical biology into a cooperating group which is unrivalled elsewhere. Today this working union stands as a model of what can be accomplished along these lines. Of this achievement Dr. Lillie was justly proud.

Professor Lillie's influence as a teacher had a far-reaching effect on students. His lectures were always characterized by a masterful precision of organization and conciseness of statement, the force of which was perhaps sometimes not fully effective owing to his soft-spoken and undramatic manner of delivery. The graduate students were not taught in the conventional way. "The student was trained to think by one who directs without seeming to do so, and was attracted first of all to the organization of the seminar and graduate courses in which the results of research, interpretations, and theories were ingeniously knit together around a central theme. Thus, the alert student was able to see how an apparently insignificant detail was concisely and cleverly woven into a concept with significant implications. The student soon learned to judge and evaluate his own performance in the seminars. The example somehow led him to strive for perfection in organization and clear thinking. The young student when he began research was to a large extent thrown upon his own resources. He found out for himself whether he was fitted to be an independent investigator. Once the problem was suggested and the way of approach briefly sketched, the student knew that results were expected. Only when a preliminary result was obtained did the student report to Dr. Lillie, and even then only when he was prepared to make a possible interpretation." (*Anat. Rec.*, **100**: 409.)

Although his scientific life was shared almost co-equally between the Marine Biological Laboratory and the University of Chicago and these interests are consequently difficult to separate, I shall confine my remarks only to those discoveries which were made solely at the latter institution. Lillie's studies on the action of

sex hormones in foetal life of cattle grew out of attempts to find an explanation of the sterility and partial inversion of the female of two-sexed cattle twins, popularly known for a century or more among cattle breeders as the free-martin. Apparently his attention was first drawn to this peculiar condition in a herd of cattle on the family farm northwest of Chicago near the village of Wheeling, Illinois. The embryological phases as worked out were made possible by the close proximity of the university to the stockyards, in which large numbers of cattle are slaughtered daily. As a student, I remember seeing him garbed immaculately in a white gown and wearing rubber gloves, examining and dissecting pregnant uteri containing young twins which the collector had rushed to his laboratory table in a breathless manner. He found that when the foetal membranes of male and female embryos are fused so that the blood vessels (especially the arteries) of the two are continuous, the female embryo is modified in the male direction. Furthermore, if no blood connection occurs both partners are normal. Without going into the complete evidence, it is sufficient to point out that no detail was overlooked in unravelling how (as he put it) "nature performed a crucial experiment" on the question of the rôle of sex hormones in embryonic differentiation of sex characters. The clear-cut and logical analysis of the free-martin published in 1917 is a model of scientific analysis and probably will stand as his most significant and enduring contribution. This work introduced biologists to the problem of sex hormones at a time when practically nothing was known about the subject. "It did much to initiate an era of intensive research in the field at large and remains as one of the important foundation stones on which a great edifice has since been built in that field." (*Anat. Rec.*, **100**: 408.)

Desiring to test and extend the application of the principles of hormone action thus gained from studies on the free-martin, he immediately initiated an extensive program of research on the isolation of sex hormones and on their rôle in controlling the expression of sex characters. For his own phase of the program he selected the Brown Leghorn fowl, a breed which exhibits what is perhaps one of the most striking examples of sexual dimorphism of plumage color. In collaboration with his research associates (especially Dr. Mary Juhn and Dr. Hsi Wang) some of the fundamental laws governing the action of hormones (estrogens and thyroxin) in the production of color patterns in feathers were discovered. Clues as to the processes involved were found to lie in the feather papilla or germ itself, a specialized derivative of the skin which exhibits many of the characteristics of an embryonic system such as axial organization, organizer-like action, and reaction gradients to hormones. Indeed, the feather papilla is an embryonic system in miniature and remains so thoroughout the life span of the bird. In elucidating these and similar problems Lillie was active almost to the end and, like his old teacher C. O. Whitman, finished on the theme of the underlying mechanisms of plumage color design in birds.

Frank R. Lillie was a part and a great part of what was highest and best in the life of the University of Chicago. His name has been built into the annals by his insight into great and guiding principles as well as by his special achievements and services. He will always remain a part of the University's treasured memories and noblest heritage.

B. H. Willier

Dr. Lillie's Relations to the National Academy of Sciences and the National Research Council

The phase of Dr. Lillie's life assigned to me for consideration has to do with his relations to the National Academy of Sciences and the National Research Council. Now I find that the records of these services are bound up in annual reports and minutes of meetings, material as dry as dust and as soulless as a stone, but there must be more in them than these records would seem to allow, for Lillie himself has said, in an autobiographical memorandum left with the National Academy, that among the offices he has held in various scientific societies he valued most the Presidency of the Academy and the Chairmanship of the Research Council. It is not possible, of course, to separate the character and personality of a man from his aims and achievements, and so we must look behind the published records to get the full story of his life, even as regards his formal relations to the organizations he served.

Lillie was elected to the Academy in 1915, the year before the Research Council was established. There is nothing in the source material available to indicate that he was very active in the Academy during the first few years of his membership. His modesty and observing character must naturally have led him to assume a waiting attitude until he became familiar with its procedures.

In the year 1919, the Society of Zoölogists chose him as one of their first representatives in the Division of Biology and Agriculture of the National Research Council, an assignment that he was to hold for four years. He was immediately made a member of the Executive Committee of the Division, the chairman of which was C. E. McClung, and was appointed chairman of two other committees, one on Coöperation and Coördination, of which I have found no record of activities, and one that must have been especially dear to his heart, on the Marine Biological Laboratory at Woods Hole. This was during the period when the Laboratory was hopeful of greater and better opportunities, when Mr. Crane had already made many of his generous gifts, including the building that now bears his name, but when it still needed a broader basis of support. In several of the annual reports of the Division in this period, a plaintive, but optimistic note is struck in such statements as this: "* * * the division and council have approved the program of the Marine Biological Laboratory and have sought energetically to secure support for this important coöperative institution. As yet this financial aid has not been secured, but it is unthinkable that it should not eventually come." The story of how it did come is told in Lillie's book on the history of the Laboratory, and I shall return to this briefly after consideration of his other accomplishments in the Division of Biology and Agriculture.

While continuing as chairman of the above committees, Lillie was chosen Vice Chairman of the Division for the year 1921–22, and became Chairman the following year. During the latter period, two important projects were brought to fruition. The first was the organization of the Union of American Biological Societies, which was later to assume responsibility for the publication of *Biological Abstracts*. The second was the establishment of the National Research Fellowships in the Biological Sciences through an initial grant of $325,000 from the Rockefeller Foundation. Lillie became Chairman of the Fellowship Board, serving as such until 1931, during the period when its policies and procedures were determined. After 1937, when

the fellowships in the physical and the biological sciences were merged into the National Research Fellowships in the Natural Sciences, he served for three years on the joint board.

To return now to the Marine Biological Laboratory: The report of Lillie's committee endorsing its program was formally approved by the Division of Biology and Agriculture and by the Executive Board of the National Research Council. Then through Dr. Vernon Kellogg, Permanent Secretary of the Council, who was also a member of the Executive Committee of the Rockefeller Foundation, the attention of the officers of the Foundation, including the President, who had been a colleague of Lillie's at the University of Chicago, was drawn favorably to the Laboratory. This led, early in 1923, to a conditional grant. By the end of the year the conditions were met, so that the Laboratory could go on with its building plans, with assurance that its endowment would be sufficient for some years to come. The building in which we are now assembled was dedicated in July, 1925.

Lillie's account of these events sounds somewhat naive in view of all the invective that has been hurled at interlocking directorates, but it evidences a wholly honest spirit without fear of criticism for taking advantage of personal and official connections in such a good cause.

Many others were, of course, actively associated in the movement to provide the Laboratory with adequate resources to satisfy the growing demands of biological research in this country. Nevertheless, it was the quiet reasonableness of Lillie's leadership as Director of the Laboratory that carried conviction and inspired confidence in all with whom he had to deal in bringing this undertaking to a happy conclusion.

While the new laboratory was under construction, conditions that were to lead to the germination of a new enterprise were taking shape. One of the persons who became interested in the Laboratory at that time was Wickliffe Rose, President of the General Education Board. Dr. Rose's interests in biology were broad; conversations were held with Lillie, and by 1925 they began to center on oceanography, in particular on the establishment of an oceanographic institution on the East Coast of the United States. It was decided that the best approach to realization of the project would be to present the plan first to the National Academy for study. This was done at the Annual Meeting in 1927, at which a motion was unanimously passed to the effect: "That the President of the Academy be requested to appoint a Committee on Oceanography from the Sections of the Academy concerned to consider the share of the United States of America in a world-wide program of Oceanographic Research and report to the Academy." Lillie's presentation of the case must have been persuasive to secure such prompt action, for the committee was appointed immediately with Lillie as chairman, and after a preliminary meeting, a liberal grant was made by the General Education Board to defray the cost of conferences and special studies on the subject. In particular, Dr. Henry Bigelow, who will no doubt say more about this, was invited to make investigations for the Committee for use in the preparation of this report, which in preliminary form was presented to the Academy at the Autumn Meeting, November, 1929. Closely following this the Woods Hole Oceanographic Institution was incorporated, and in February, 1930, the Rockefeller Foundation made to it a grant, ultimately amounting to three million dollars, for construction and equipment of laboratory and ships and endowment of the Institution. Lillie became

its first President, serving until his retirement in 1939. At the same time a grant was made to the Trustees of the Bermuda Biological Station to meet the conditions of a gift from the Colonial Government. These two grants enabled the Station to proceed with its plans for development. In recognition of his services to oceanography the National Academy in 1940 awarded him the Agassiz Medal.

His three main accomplishments of the decade 1920–30 in connection with the Academy and Research Council, i.e., the establishment of the fellowships in the biological sciences, the placing of the Marine Biological Laboratory on a secure financial foundation with greatly increased facilities for work, and the foundation of the Oceanographic Institution, add up to more than most men can look back to in an equal length of time. In promoting these activities, he was either acting as agent for the Academy and Council, or was using quite legitimately the prestige of these organizations to further the purposes of other institutions he had at heart. All of this was done while administering a large department at the University of Chicago, serving as director of the Marine Biological Laboratory and engaging in research of high quality.

Lillie served the Academy and the Research Council in various other ways. He was chairman of the Daniel Giraud Elliot Medal Committee, 1926–29, and of the Murray Fund Committee, which awards the Agassiz medal, 1929–32.

Shortly after the Committee on Problems of Sex was set up in the Division of Medical Sciences with funds for grants-in-aid on a liberal scale, Lillie was appointed a member. He had a few years before, when little was known of sex hormones, completed a remarkable piece of research on the development of the freemartin, the female member of a heterosexual twin-pair in cattle, which is regularly sterile and gives evidence of partial sex inversion. His explanation of this phenomenon gave an entirely new turn to the then prevalent views on sex differentiation and constitutes one of the foundation stones of the imposing edifice that has since been erected in this field. Lillie thus brought to this important committee mature judgment, based on experience, and became one of its most valued members.

In 1933, when our country was in the midst of its most severe economic crisis, the Government turned to the Academy and Research Council for assistance, and the President of the United States appointed by executive order a "Science Advisory Board with authority, acting through the machinery and under the jurisdiction of the National Academy of Sciences and the National Research Council, to appoint committees to deal with specific problems in the various departments." All but two members of the Board were also members of the Academy and Lillie was included in its number. However, a misunderstanding of the responsibilities of the Academy for the appointment of its own committees led to an unfortunate controversy between the administrative officers of these two bodies. The board worked without regard to this and had brought in two long and valuable reports by the time of its discharge in 1935. Nevertheless, the differences in opinion between the officers of the Academy and the Council had been so sharply drawn, that when the time arrived for a change, it seemed to those concerned that the best way out would be to effect a personal union between the presidency of the Academy and the chairmanship of the Research Council. Lillie, because of his personal qualities and administrative skill, was unanimously chosen to fill this dual post. He went about his task with quiet determination, and by the end of his first year of office the two organizations were working so smoothly that he felt he could relinquish the chair-

manship, retaining the presidency for a full term of four years. One of the administrative staff writes of this period: "I always thought that through the force of his integrity and high esteem in the scientific world he was able to create in the minds of a number of other opposing members of the Academy a better understanding of the place of the Council in the Academy's structure." Those who succeeded him in two offices owe him a debt of gratitude for his statesmanship.

As President of the Academy, 1935–39, Lillie showed his usual human qualities. There were no spectacular happenings, but real progress was made in several directions, such as the establishment of the Central Purposes Fund through a grant from the Rockefeller Foundation, and the inauguration of the Pilgrim Trust Lectures. The former is a much needed fund for financing conferences, government relations and international projects; the latter, now unfortunately in abeyance for lack of funds, involved an exchange of lecturers between the National Academy and the Royal Society of London.

The subject of my address has necessitated emphasis on Lillie's administrative work, but I should not wish to close by leaving with you the impression that I thought this to be his most important contribution. With all of his exacting duties as director, chairman, professor and dean, he never lost interest in the progress of research. Not only this, he also never gave up engaging in research himself, and it was research of a high order—important, original, stimulating and carried out with a pride of workmanship that could well serve as a model for his many students. For this his memory will as long endure as for his many distinguished contributions to the organization of Science.

Ross G. Harrison

Dr. Lillie and the Founding of the Woods Hole Oceanographic Institution

The outstanding facts all have been set down by Lillie himself, in the book he wrote before he died. If you want to see the Oceanographic Institution you have only to look across the street. What I do propose is to try and tell you what kind of a man I found him during the years of our close friendship and intimate association, not about his scientific achievements.

Perhaps the characteristic which always impressed me most was his almost uncanny capability of sifting out pertinent facts in a situation, and of reaching a conclusion in a perfectly objective way, without personal bias. The result was that almost anyone else, thinking equally objectively, was almost bound to come to the same conclusion. The deliberations of the Oceanographic Institution are an excellent example. During the ten years of his presidency many questions came up regarding scientific activities, general policy, and social relations with the community where it is situated. From the very beginning he insisted that the one criterion on which decisions must be based was whether a proposed course of action would or would not likely meet the basic aims of the Institution, i.e., encourage the study of the sea. If it seemed that it would, we voted yes; if not, we voted no. And I can't remember a split vote by the Trustees during all the years of his presidency.

I do not know when the idea of an Oceanographic Institution at Woods Hole first formed in his mind, though I have no doubt that this happened long before any of us heard about it. At any rate, he did not move in the matter until the time

was ripe. This appeared to be the case during the late '20's when the results of the Gulf of Maine investigations had been published, when the Navy was interested, when work was actively being done on the Pacific coast, when, in a word, oceanography appeared to be stirring in the U. S. The General Education Board, then in existence, was interested in supporting the broad range of scientific activities, and I have no doubt it was thanks to Lillie that the desirability of supporting oceanography was discussed here at Woods Hole during the conferences of 1927. He, I think, saw more clearly than anyone else that recommendations of the National Academy would have much more influence than would any backing by individuals. And evidence that such was the case was to be seen in the fact that the Rockefeller Foundation did endow the Oceanographic Institution, upon the recommendation of the Academy.

The budget of the Institution is another example of his foresight. As early as 1927, he had arrived at the tentative figure of 3 million dollars as the amount that would be required to set up and run an establishment such as he visualized. And when we sat down to figure out definite costs for building, for ships and for operations, it all added up to just that amount. A word more about the ship. I had been working on boats of one sort or another for many years and felt that an oceanographic institution could not be operated to any advantage without a seagoing vessel. When I brought this up with Dr. Lillie I was somewhat discouraged, for at first he did not seem very receptive. But it soon developed that he was in full sympathy and had been from the beginning, but that he realized, better than I, that it would be difficult to put the idea over. At that time the Foundations were quite accustomed to being asked for money for buildings, for personnel, and for libraries, but not to being asked for $200,000 for an oceanographic ship. In fact, no private institution in the world possessed such a vessel at that time. He knew very well that the only way he could put the idea over was for someone who had had practical experience with oceanographic work at sea to provide him, as ammunition, not only with a thought-out statement as to why a ship was needed, but also with all details as to the type of vessel needed, its size, its cost of construction, and its cost of operation. With this in hand, he could proceed. The result was the Atlantis.

I think I have come to the end of my fifteen minutes, but want to leave with you one other thought, and that is how close it was to Frank Lillie's heart that the Oceanographic Institution should not only be a credit to science and to the National Academy, but that it should also be a good thing for Woods Hole. I think it has been, and I hope that the rest of you think so too.

H. B. Bigelow

Frank R. Lillie and the Marine Biological Laboratory

Mr. President, Ladies and Gentlemen: I suppose you are all familar with that story of Edward Everett Hale, "My Double and How He Undid Me." This double always followed other speakers with the single sentence, "So much has been said and so well said that I need say nothing more!" I am in that position, only more so, having not only one but three predecessors on this program. Those who have gone before me have told so much of what I expected to say that I think it may be well for me to cast aside my manuscript and recite as well as I can from memory many of the contacts which I have had with Dr. Lillie during all the fifty-

five years that he spent at this institution. No other person associated with the Woods Hole Laboratory has ever devoted himself so continuously and for so long a period to it. He came here, as you have already heard, from the University of Toronto where he graduated with the Bachelor of Arts degree in 1891. One of his favorite teachers there was R. Ramsey Wright. It is a significant fact that Wright had been elected a member of the Corporation of the M.B.L. in 1890. Lillie graduated in 1891. The new Woods Hole Laboratory was relatively unknown, and undoubtedly young Lillie heard of it from Prof. Wright, who became a trustee of the laboratory in the same year that Lillie came here. He was classified as an investigator receiving instruction in the report of the laboratory for the year 1891. Lillie came here at the fourth session of the laboratory. It had been going only three summers before he came, and every summer from 1891 until 1946 he had part in the work that went on here. Last summer, unfortunately, he was prevented by his fatal illness from coming. That was the first time in all those fifty-five years, which covered his entire professional life, when he was absent.

When he arrived here in 1891 he at once saw Prof. Whitman, who had the custom of at once setting his students to work on a research problem, trusting that they would get their education in the course of their research. Whitman that summer was studying leeches from the fresh water ponds in the vicinity of Woods Hole. (In those days Woods Hole was spelled Woods Holl and when the Post Office Department changed the spelling to "Hole," Whitman refused to accept the new spelling, saying that he had named some of his new species "hollensis" and that established scientifically the name Holl.) Lillie went with Whitman on some of his trips to the ponds of the vicinity and they found the pond mussel, *Unio*, bearing eggs and embryos which were caried in the gills. Whitman proposed that Lillie undertake the study of the embryology of this form, and this he did with such effect that he won the praise of all in the laboratory. At that time the study of the individual cleavage cells and their development was occupying the attention of several of us, and Lillie's work added very remarkably to the importance of this work. One of the important results of his study was the proof of the adaptation of cleavage to the future morphology of the animal that developed from the egg. This work was of such note that it led to his nomination as a member of the Corporation that same year, and to his appointment to a fellowship in Morphology at Clark University, which had been established only a few years before. Whitman was Professor of Morphology there, and a good many of Whitman's students and associates at Clark were at Woods Hole in 1891. Among them were: Bristol, Bumpus, Donaldson, Mead, Jordan, MacMurrich, Watase and Wheeler. All were either members of the faculty or graduuate students at Clark. They were a remarkable group, testimony that Whitman knew how to choose men. In the autumn of 1891 Lillie went with this group back to Clark. Conditions there were not ideal. The man who had founded the institution still lived there and felt that he ought to keep his hands on it. Whitman complained of petty annoyances such as the query, "What is the need of using so much alcohol in this laboratory?"

At that time the great University of Chicago had been re-established by the gifts of John D. Rockefeller. President Harper was looking around for faculty members—leaders. The brother of President Harper was an intimate friend of Prof. Mall, the anatomist, and Mall was instructed to sound out the members of the faculty of Clark, to see if they would go to Chicago. With very great success this was done. The entire Department of Zoölogy went to Chicago and, in addition,

Mall, Donaldson, Michelson, Neff and Bolza also went. Lillie, of course, went with them. He was fellow in Animal Morphology the first year at Chicago. The second year he was appointed reader in Embryology. The third year he received the Doctor's degree, his thesis being on the Embryology of the Unionidae. In the meantime he had come every summer to Woods Hole and taught in the courses, particularly in Embryology. His ability as organizer and teacher was recognized, and he became Assistant Director of the laboratory in 1900 and was elected Director in 1908.

In the early days members of the scientific staff generally served without salaries and at the end of the summer Whitman very often went around and said, "We must have some money to pay the janitors and collectors. Can you help us?" Some of us became life members of the Corporation in that way. Those who lived long have found that bargain profitable. We have been members of the Corporation and have received the BIOLOGICAL BULLETIN for 50 years or more, merely because we went without salary which might have been paid us as teachers. Income of the laboratory in those days was from student fees, the supply department, and from minor gifts. Whitman had little fear of deficits. He was more interested in ends than in means. I said to him on one occasion, "That is going to cost a mint of money." "Great God," he said, "What is money for?" Consequently, many of the Board of Trustees, consisting of conservative Bostonians, were distressed. They passed orders with regard to not running into debt, which were disregarded by the Director. This and a system which may be characterized as "absentee landlordism" led to a break with the first board of trustees. You know how in the summer of 1897 the members of the Corporation, who were working in the old wooden building, chartered some cars on the railroad and went to Boston, 80 strong, to hold a legal corporation meeting and revise the By-Laws. At that meeting reorganization of the laboratory took place. After that, meetings of the Corporation and Trustees were to be held in Woods Hole in the summer, and not in Boston in the winter. This gave the laboratory workers a wider representation than before. It became more thoroughly national. But this did not help out a bit in the matter of finances. We were poor as ever; perhaps even worse off. Many doubted the ability of men of science to manage business affairs. I remember hearing many say that American universities and colleges had found it necessary to have a board of trustees for purposes of raising funds and to leave the faculty free to deal with subjects with which they were more familiar.

In 1901, nine years after the founding of the University of Chicago, President Harper proposed that a board of Trustees, composed of men of financial standing, be formed to run the M.B.L. and to secure funds to enable it to run without such difficulties. But the scientists in charge of the laboratory were jealous of losing control and feared that this would tie up the M.B.L. with the University of Chicago. And so it was not approved.

In 1902 the Carnegie Institution of Washington was established. The President of the M.B.L. was H. F. Osborne and one of the important trustees was E. B. Wilson. They both knew officers of the Carnegie and were asked to present to them certain projects. They proposed the support of the M.B.L. and it was accepted by the officers of the Carnegie with a certain amount of enthusiasm. But they found that they could not make it their Department of Marine Biology without owning the Laboratory outright. There was conflict of opinion among the Trustees as to whether we should surrender possession. Finally, on August 12

in that year (1902), the Corporation and Trustees of the M.B.L. voted by a great majority (60 to 3) to deed the laboratory to the Carnegie Institution. Whitman was broken-hearted; Lillie, also. Whitman wrote a stirring appeal, later published with the approval of the chairman of the Carnegie executive committee, entitled, "The Impending Crisis in the History of the Marine Biological Laboratory." The Carnegie then withdrew the proposal to take possession of the laboratory and instead proposed to give $10,000 a year for three years to help the laboratory get started on a better course. In all these crises Lillie stood with Whitman—in the first revolt of 1897 and in that of 1902. And although feeling ran high and there was a good deal of personal dissension, there was never any condemnation of the action of Dr. Lillie. He was so reasonable and so unemotional, that there was no question but that people could go along with him.

After this Prof. Whitman gradually withdrew from the laboratory. He was not present here in 1904 and 1905. His house on Centre Street was burned down in the winter of 1906 and his friends rallied and bought the property, rebuilt it, and urged him to come back. He did come back in 1906 and again in 1907, but resigned in 1908, and never returned until his body was brought here for burial in 1910. Lillie had been since 1900 Assistant Director, and when Whitman retired in 1908, Lillie, of course, was the man chosen to succeed him. From that time on, the affairs of the laboratory began to improve. The improvement was multifarious; it was not merely financial. Drew became associated with Lillie as Assistant Director in 1908, and his appointment showed Lillie's good judgment in choosing men.

In the meantime Lillie had secured the active interest of his brother-in-law, Mr. Charles R. Crane, in support of the laboratory. Mr. Crane was elected President in 1904 and continued in that office for twenty years. During all that time his gifts increased to $20,000 a year, in addition to important gifts for real estate holdings. In 1912 Mr. Crane authorized construction of the Crane building which cost at that time, before the first World War, $111,000. But the needs of additional space in a substantial building, equipped for work in bio-physics and bio-chemistry, as well as for library, lectures and administration, were especially urgent after the first World War, and plans were made for another new building. Lillie was the leader in this movement, ably supported by Dr. Drew and other members of the trustees, and with the promised support of Mr. Crane. There was also special need for increased endowment, and the trustees voted to appeal to the Rockefeller Foundation and the Carnegie Corporation for one million dollars to meet these needs. Fortunately the M.B.L. and Dr. Lillie were well and favorably known to the officers of these foundations, and in 1922 the Rockefeller Foundation voted to authorize a half million dollars for these purposes on condition that another half million be secured from other sources and that Mr. Crane capitalize his annual gifts of $20,000. The Carnegie Corporation also voted to approve a gift of $100,000 for endowment, provided that a total sum of one million dollars was secured. For nearly two years the laboratory lacked $400,000 of making up its half million to secure the half million for the Rockefeller Foundation. Finally at the end of 1923 Mr. John D. Rockefeller, Jr. requested Simon Flexner to investigate the needs of the M.B.L. and to report to him. Flexner came down to see how funds in hand were used, and whether we were worthy of increased support. I remember that at the conference here, in addition to Flexner there were Lillie, Morgan, Loeb, Wilson and myself. We all presented the case eloquently and effectively, it seemed to me,

except Loeb, who said, "I am against another building. If we grow and have a big institution, we will spend all our time taking care of it." Morgan said he thought the wooden buildings were all right. I was shocked and I looked across at Lillie. He also was shocked. Then I looked anxiously at Flexner and he just winked one eye at me. I then realized that he knew the critical nature of Loeb and Morgan, and I felt relieved. Flexner went back to New York and made a favorable report, and Mr. Rockefeller personally gave the remaining $400,000 to complete the million dollar fund. Mr. Crane then endowed his annual gift of $20,000 with bonds of a par value of $405,000, and in addition agreed to meet all costs of the new building above $500,000; this ultimately amounted to $221,000. The total results of this campaign were, therefore, for the new building $721,000, for endowment $905,000, or a total of $1,626,000.

In 1924 the General Education Board gave $50,000 for the purchase of books for the Library and in 1929 it gave $200,000 for endowment of the Library. The Rockefeller Foundation gave $110,000 for the five-story library extension in 1941–42, and the Carnegie Corporation $25,000 for the purchase of books and serials. Thus the total sum of nearly $300,000 for the Library was secured from these sources. In the meantime Dr. Lillie had brought to the attention of Dr. Wickliffe Rose, President of the General Education Board, the pressing needs of safe and comfortable living quarters for workers at the laboratory, and in 1926, $250,000 was appropriated for the brick dormitory and apartment house.

Dr. Lillie wrote in his summary on the "Material Growth of the Laboratory" in his last book, *The Woods Hole Marine Biological Laboratory*, "The land, buildings, library and equipment presented expenditures of about $1,980,000 up to 1941. The major and minor endowment funds amounted to about $1,125,000; the value of the interest of the Laboratory in a biological supply house and a public garage on a very conservative capitalization of income amounts to at least $280,000. The total resources are thus $3,385,000, and there is no indebtedness." In 1900, when Dr. Lillie became Assistant Director of the laboratory, its entire assets including land, buildings, library, equipment and endowment had an estimated value of $35,000. Forty-one years later, when he retired from the Presidency, those assets were one hundred times greater. Many members of the Staff, Trustees, Corporation, and many devoted friends and organizations contributed to this great growth, but the leader in gaining the confidence and support of financiers and of great foundations was Frank R. Lillie.

His important contributions to biological research and education, his leadership in the conception and development of the Woods Hole Oceanographic Institution, his services in connection with the University of Chicago and the National Academy of Sciences were major achievements in a notable career, but I think the one thing in which he took most satisfaction was his part in the development of the Marine Biological Laboratory. Here his professional life began, here his research work first received world-wide recognition, here his ability in administration and organization was first demonstrated. Here he met the research student who became his devoted wife and helpmate, here he established his family and his hospitable summer home, here his thoughts and affections were always centered, and it is most fitting that he was buried here, where so many of his old friends and associates of the Marine Biological Laboratory are buried, almost within sight of the institution which he so much loved, and which shall be his enduring monument.

E. G. Conklin

SOME OBSERVATIONS ON THE GOLGI MATERIAL IN THE LARVAL EPIDERMAL CELLS OF DROSOPHILA MELANOGASTER

W. SIANG HSU

University of Washington, Seattle, Wash.

Introduction

In my attempt to make a general survey on the morphology and behavior of Golgi material in a number of tissues of the larvae of Drosophila melanogaster, I have encountered in the larval epidermal cells some rather interesting phenomena which form the substance of this paper. These phenomena, besides confirming the facts and conclusions reported in my previous papers dealing with Golgi material in the larval tissues of the same fly (1947, 1948), seem to point rather definitely to a hitherto unreported function of the epidermal cells of Drosophila larvae—that of internal secretion.

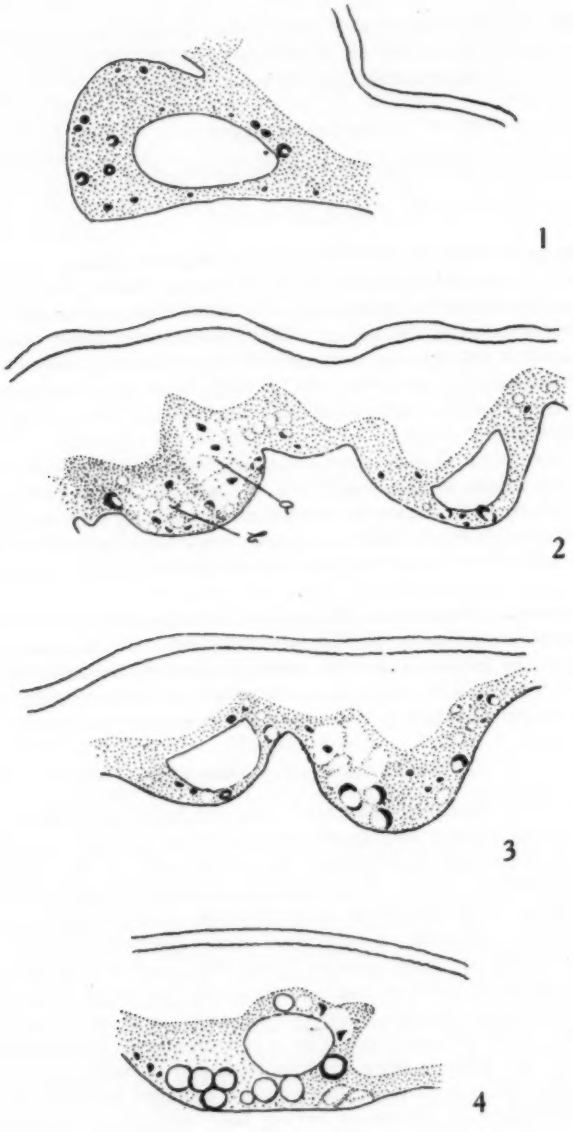
The larvae used for this study were from "wild flies." They were raised in the same cultural conditions as those used for the mid-gut study (1947). The undesirable effect of crowding the larvae was avoided by placing four or five females for only 24 hours in each bottle of 100 c.c. capacity containing approximately 10 cc. of food. Besides, only those which first reached the desired age in a fresh bottle were fixed.

For Golgi material observations, Koletchev and Mann-Kopsch techniques were employed; but the Golgi bodies in the epidermal cells, as in practically all other tissues studied in the larvae of this fly, seem to show up far better in the Mann-Kopsch slides. These slides were so much relied upon for critical observations that all figures except Figure 5 in this paper were drawn from them.

Observations and Discussion

In a section taken almost at any point along the length of a larva, most of the epidermal cells would present such appearances as shown in Figures 2 at a, 7, and 8. The cytoplasm in these cells is extremely vacuolated; and, in the vacuolated areas, apparently homogeneous bits of Golgi material of various shapes are observable. Careful focussing on these vacuolated areas, however, would usually show that they are the results of a confluence of individual small spherical globules. Evidences supporting such a view are easily obtained, once the observer is made aware of the situation. The apparent unity of many of these big and irregularly-shaped vacuolated areas would often resolve, upon careful study, into many individual small spherical globules, each still surrounded by a very thin layer of cytoplasm (Figs. 2 at b, 5 at a, 6). So, when a large irregularly-shaped vacuole is seen in a cell, it may be in a stage wherein its contributing small globules have all lost their individuality, the thin layer of cytoplasm having been withdrawn from around them (Figs. 7, 8). However, it may also be in a stage when the individual small compo-

PLATE I



nent globules can still be made out unmistakably (Figs. 2 at b, 5, 6). The smaller globules found within a large vacuolated area are of various sizes; and it should be noted that their sizes correspond very closely to those of the globules observed elsewhere in the cytoplasm where the formation of a large irregularly-shaped vacuole cannot as yet be easily suspected (Figs. 2, 3, 6). This is pointed out as an evidence, aside from what is indicated by the relation between the globules and the Golgi material, which will be discussed presently, that the globules both within and without the large vacuolated areas are of the same category of entities, and therefore they should have a similar origin.

Upon examining a cell at a stage of vacuolation as shown in Figures 2 at a and 7, it would be difficult to see any relation between the bits of Golgi material and the big vacuoles. But the condition as illustrated in Figure 6 reminds me rather strongly of the behavior of Golgi material in cells actively engaged in synthesizing secretion, such as have been observed in the cells of the mid-gut epithelium (1947) and those of the salivary glands of a Drosophila larva (1948). The appearance of the Golgi material in its relation to the spherical globules depicted in the same figure recalls its condition seen in the cells of both the tissues mentioned above when the individual secretion granules or droplets have grown to such a size as to be ready to be freed from the confining Golgi shell. The granular appearance of the Golgi rim around the globules is characteristic of the Golgi material when its separation product is ready to be released and itself about to break into bits of irregular shape, each of which is presumably capable of starting another cycle of secretion when proper conditions again prevail.

In addition to the observations mentioned above, my experience with the behavior of Golgi material in the glandular cells of Drosophila larvae inclined me to believe that two or three pieces of Golgi material seen in the cell represented by Figure 2 also showed familiar signs of being in the act of separating some kind of secretional material in their interior. These observations led me to a search in a large number of slides for more definite evidence in that direction. My effort was rewarded with a number of cells such as represented by Figures 3 and 4. In these

---

Explanation of Plates

All the figures of the two plates are camera lucida drawings at a magnification of approximately 2200 ×.

Golgi material is represented as black dots, crescents or rings; secretion globules, as circles of broken lines; and cytoplasm, as stippled areas. A nucleus, whenever included in a figure, is indicated by an unstippled area marked out with a solid line. The epidermal cells are depicted in the figures as being over-laid with two layers of cuticula.

Plate I

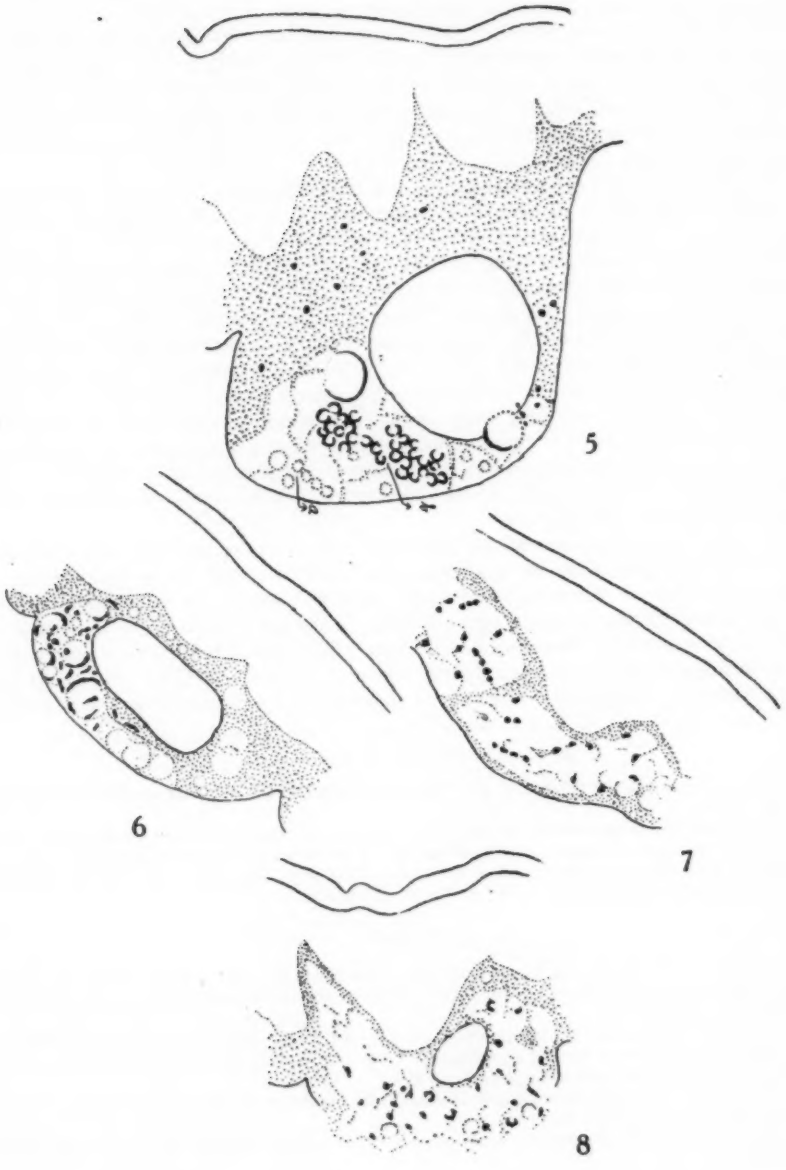
Figure 1. A cell showing in the cytoplasm some pieces of Golgi material which are apparently homogeneous and some in each of which a light center has become visible; no free secretory globules are yet observable.

Figure 2. Two cells: the one to the right showing a number of free secretory globules in the cytoplasm; the one to the left showing at b a few free globules about to be fused to produce in the cytoplasm a condition such as depicted at a.

Figure 3. Two cells showing practically all the stages of the development of a secretion globule in relation to Golgi material.

Figure 4. A cell showing the unmistakable origin of the secretory globules in the Golgi material.

PLATE II



two cells, a Golgi-material origin of the spherical globules is unmistakable. Figure 3 shows practically all the stages of the development of a secretion globule in relation to Golgi material, from apparently homogeneous bits of Golgi material to mature globules lying free in the cytoplasm.

Thus, regarding the origin, growth and releasing of the secretion globules in relation to Golgi bodies, the situation as found in the epidermal cells may be summed up as follows: Inactive pieces of Golgi material appear homogeneous; but when secretory synthesis has proceeded sufficiently far, there becomes visible one light area in each Golgi body. This area has been interpreted, on evidences which have been reported in a previous paper (1947), to be a globule of elaboration product viewed through a layer of Golgi material. As the globule increases in size, the light area in most of the Golgi bodies becomes less and less colored as a result of the continual thinning of the overlying layer of Golgi material on the surface of the globule. A time will eventually be reached when the Golgi shell will no longer be able to contain the enlarging globule within it and will mechanically break into small irregular pieces, releasing its contents into the cytoplasm. This series of changes almost exactly duplicates what has been seen in the other larval tissues of Drosophila definitely known to have a glandular function (1947, 1948).

What I consider as most instructive and significant, however, are strips of cells, usually five to six in number, which have often been observed with their cell membrane broken but with their nuclei and part of their cytoplasm still intact (Fig. 8). These cells remind one most vividly of the method of discharging their secretory product seen in the larval mid-gut epithelium and salivary gland cells of this same fly (1947, 1948). When one adds to this situation the relation observed to be existing between the Golgi bodies and the globules which they elaborate, as pointed out in the preceding paragraph, the suggestion becomes more than probable that in the Drosophila larvae, the epidermal cells may also serve as internal secretion glands. Thus, Figures 1 to 7 may be taken as showing graphically the various stages of secretory synthesis which an epidermal cell passes through—from a stage wherein the cytoplasm contains no free secretory globules but numerous Golgi bodies (some are visibly homogeneous while others show a light center) to a stage in which the cell is extremely vacuolated due to the confluence of a large number of secretory globules elaborated and set free in the cytoplasm by the Golgi bodies. Figure 8, finally, illustrates the merocrine method of discharging secretion by the cell. Having failed to see any replacement cells in the epidermis, I assume that after having

PLATE II

FIGURE 5. A cell showing the vacuolated condition of the cytoplasm near the basement membrane. A few unfused globules are still visible within the vacuolated area at a; a group of Golgi bodies all at about the same stage of secretory synthesis are seen at b. Note the two large globules not yet freed from their respective Golgi shell and also the bits of apparently homogeneous Golgi material in the cytoplasm near the cuticula end of the cell.

FIGURE 6. A cell showing the Golgi shells around the globules already or about to be broken into small pieces which have the rugged surface and irregular shapes characteristic of apparently homogeneous and inactive bits of Golgi material.

FIGURE 7. A cell showing its cytoplasm extremely vacuolated with apparently homogeneous bits of Golgi material embedded on strands of cytoplasm; at the right end of the cell may yet be seen some globules in the process of fusing.

FIGURE 8. A cell with its cell membrane broken discharging its secretion and also a portion of its Golgi material into the body cavity of the larva.

discharged its store of secretion, each cell is capable of repairing itself and starting another cycle of secretion when proper conditions are again present. This is the situation which prevails in both the mid-gut epithelium and the salivary gland cells.

It is interesting to record that it was after I had reached the conclusion that the cells of Drosophila larvae may serve as internal secretion glands that my search into the literature for some supporting opinion led me to a paragraph by Wigglesworth (1934) in his study on ecdysis in Rhodnius, which I quote: "The histological evidence therefore favours the idea that the corpus allatum is responsible for secreting the moulting hormones which must be derived from the growing cells themselves, and this raises the question whether the general epidermal cells may not be responsible for the initial moulting hormone. This possibility cannot be entirely excluded; but the epidermal cells are not innervated, and it is therefore probable that any hormones they secrete appear only when their own growth has been stimulated by the hormone from the head." This is the first reference to the epidermal cells as internal secretion organs which has come to my knowledge, although it must be admitted that my search in the literature in that regard is not an exhaustive one. However, in quoting Wigglesworth, I do not claim that my observations prove that the epidermal cells are "responsible for the initial moulting hormone" in Drosophila larvae. Needless to say, this question merits more particular examination. I only claim that according to my material it is difficult to dismiss the idea that the epidermal cells of a Drosophila larva are capable of secreting some substance and that the secretion is discharged directly into the body cavity of the larva.

Summary

1. On the strength of observations set forth in the following paragraphs, it has been concluded that the epidermal cells of Drosophila larvae seem to act as internal secretion organs, at least at the age when the larvae are about one day before pupation.

2. The relation of the Golgi bodies to the globules, both inside and outside of the Golgi bodies as observed in the epidermal cells, has been found to be the same as what has been established in cells definitely known to be of a glandular nature in the larvae of this fly. In each piece of Golgi material, a single droplet is seen to make its first appearance and gradually to increase in size, eventually breaking free from the confining Golgi shell. It seems to be the normal procedure for the free separate secretory droplets to coalesce to form big vacuoles; and their further confluence gives to the cells in advanced secretory synthesis an extremely vacuolated appearance.

3. Many epidermal cells have been found with their cell membrane broken, thus releasing their secretion product into the body cavity of the larva. The apparent healthy condition of the nuclei of such cells and the absence of replacement cells in the epidermis would point to a merocrine mechanism of secretion in this case.

LITERATURE CITED

Hsu, W. S., 1947. On the cytoplasmic elements in the mid-gut epithelium of the larvae of Drosophila melanogaster Meigen. *Jour. Morph.*, **80**: 161–194.

Hsu, W. S., 1948. The Golgi material and mitochondria in the salivary glands of the larva of Drosophila melanogaster. *Quart. Jour. Micr. Sci.*, **88** (in press).

Wigglesworth, V. B., 1934. The physiology of ecdysis in Rhodnius prolixus (Hemiptera). II. Factors controlling moulting and "metamorphosis." *Quart. Jour. Micr. Sci.*, **77**: 191–222.

THE ROLE OF THE SINUS GLANDS IN RETINAL PIGMENT MIGRATION IN GRAPSOID CRABS

RALPH I. SMITH

Department of Zoology, Univ. of California, Berkeley 4

Introduction

Among the several functions ascribed to a hormone or hormones produced by the sinus gland of the crustacean eyestalk is that of controlling the migration of the retinal pigments. Evidence for this function has been presented chiefly by Bennitt (1932a, b), Kleinholz (1934, 1936), and Welsh (1939, 1941) and is well summarized in the reviews of Kleinholz (1942), Brown (1944), and Panouse (1947). Briefly stated, the compound eyes of decapod crustaceans typically possess three sets of pigment cells: distal melanophores forming a sleeve about each ommatidium, proximal melanin contained within the retinular cells (photoreceptive cells, proximal pigment cells), and reflecting pigment cells located among and beneath the retinular cells. In day-adaptation the proximal and distal melanins approach each other so as to surround the sensitive rhabdomes, while the reflecting pigment may be shifted proximally beneath the basement membrane of the retina. In night adaptation the proximal and distal melanins move apart in such a way as to leave the rhabdomes exposed to light from all sides, while the reflecting pigment is exposed to form a reflecting layer at the bases of the rhabdomes, visible as a reddish area of "glow" in the dark-adapted eye. The relative extent of movement of the three types of pigment differs in detail in various species of crustaceans. Attempts to demonstrate nervous control of retinal pigments in crustaceans have been unsuccessful, while the possibility that these cells are independent effectors is not supported by the available evidence. In particular, the marked diurnal movements of retinal pigments exhibited in the eyes of many crustaceans under conditions of constant darkness or of constant illumination would seem to rule out independent response to illumination as anything but an incidental factor in the normal process of pigment migration. Positive evidence for a hormonal control is based upon the discovery by Kleinholz (1934, 1936) that extracts of light-adapted *Palaemonetes* eyestalks injected into dark-adapted animals in darkness caused the distal and the reflecting pigments to move to the light-adapted position. This work was confirmed on *Cambarus* by Welsh (1939), who found that the proximal pigment was likewise induced to migrate to the light-adapted position if sufficiently strong injections of eyestalk extract were used. It has generally been assumed that the sinus gland is the source of the retinal pigment activator, and Welsh (1941) has shown that aqueous extracts of isolated sinus glands are capable of causing typical pigment migration when injected into dark-adapted crayfish. As will be reported below, similar results may be obtained in grapsoid crabs.

While the evidence for a hormonal control of crustacean retinal pigments is generally convincing, this concept has been based upon procedures which have not

included one of the classical tests of endocrine function, namely, the removal of a suspected organ to produce characteristic symptoms, followed by the injection of extracts or the implantation of the organ to restore the normal conditions. Furthermore, most experimental work has been carried out with macrurans. Accordingly, it was felt that a further study of brachyurans would be of value, and that sinus gland extirpation should be made the main line of approach to the problem.

If the sinus gland is the source of retinal pigment activating hormone, its removal would be expected to stop diurnal pigment migrations and to maintain the eye in a dark-adapted state. Continued display of retinal pigment migration after sinus gland removal would, on the other hand, indicate that this organ is not the controlling factor or at least not an indispensable link in the process. Welsh (1941) has given evidence that the control of diurnal changes in retinal pigments involves central nervous activity, possibly affecting the release of hormone by the sinus gland, but the possibility that this effect can be mediated in the absence of the glands has not been investigated. The present paper reports attempts to clarify the role of the sinus glands in retinal pigment migration by means of operative sinus gland removal.

Material and Methods

A. *Animals*

Three species of grapsoid crabs common in the San Francisco Bay area have been employed: *Hemigrapsus oregonensis, H. nudus,* and *Pachygrapsus crassipes.* These animals are hardy, possess well-defined sinus glands, and show a marked "glow" in the eyes in darkness at night. With the exception of the work of Bowman (M.A. Thesis, U. C., Berkeley, Calif., 1948) no previous study of the pigmentary changes and endocrine functions of this group of crabs has been made. In order to reduce possible complications associated with egg-bearing, chiefly males have been used in the work, although enough females have been observed to indicate that their pigmentary responses are the same as displayed by the males. Animals were selected for size, since those less than 17 mm. in carapace width were too small for convenient sinus gland removal, while those exceeding 30 mm. in width were not only too large and active for easy handling under the dissecting microscope, but also possessed such thick and pigmented exoskeletons as to render difficult the observations on body chromatophores sometimes carried on concurrently with studies of retinal pigments.

All animals were kept in shallow water in individual covered glass dishes, were fed fresh liver, clam, or crab meat and had the water changed every 3 or 4 days.

B. *The preparation of active extracts*

Sinus glands and other organs to be tested for retinal pigment activating effect were taken from animals of approximately the same size as the recipients, so that dosage could be estimated in terms of a fraction of the organ in question. Recipient crabs were measured or weighed before an experiment, and a group of donor animals selected so as to have a slightly greater average width or weight than the recipients. Eyestalks were removed, split open in crab perfusion fluid, and the chain of optic ganglia with the attached sinus gland teased free with fine forceps. The sinus gland was detached and removed to a drop of distilled water in a covered one-inch Syracuse dish. The medulla terminalis was similarly placed in a second dish, and the

group of three more distal ganglia (medulla interna, externa, and lamina ganglionaris) in a third dish. Other tissues extracted included brain, which in these crabs has about twice the mass of the medulla terminalis, thoracic ganglionic mass, and claw muscle. The respective tissues, when a sufficient amount had been collected, were ground with rounded glass rods, although the small sinus glands ordinarily escaped thorough crushing. Crab perfusion fluid was added to the dishes with a 1 cc. hypodermic syringe, stirred, and then taken back into a marked syringe for transfer to a small test tube. The volume of fluid used being known, a further quantity of perfusion fluid sufficient to dilute the suspension to the desired degree was added to the Syracuse dish, and likewise transferred with the marked syringe to the test tube. Ordinarily, 10 or 20 sinus glands, medullae terminalis, and distal ganglia were used, and made up to give extracts containing the equivalent of 10 organs in one cc. Brains were diluted to 5 per cc., while thoracic nerve mass and claw muscle were dissected out in quantity judged to be a little greater than the mass of brain tissue used. After transfer to test tubes, all extracts were heated in boiling water for 10 minutes, cooled, and used unfiltered. Injections were made at the base of a walking leg, the quantity injected being 0.05 cc., using for each extract the same syringe used in handling that material in preparation. Extracts stored in a refrigerator retained their potency for several days.

C. *Determination of the relative size of extracted organs*

In order to arrive at a dilution factor, so that extracts could be diluted to contain a bulk of tissue comparable to that present in an extract of sinus glands, there were dissected out in turn from each eyestalk of four crabs the sinus gland, the medulla terminalis, and the group of three distal optic ganglia. These organs from each eye were placed on a haemocytometer slide in crab perfusion fluid and compressed under the cover glass (0.1 mm. clearance). Camera lucida tracings on good quality graph paper were made, the outlined areas being cut out and weighed in groups. A test made by cutting out squares of known area showed that the combined errors in cutting and in thickness of the paper did not exceed 1.5 per cent, whereas variations in dissection were large. In Table I the relative weights of the separately dissected organs are tabulated, showing the wide variation that may be expected in removing

TABLE I

Relative sizes of sinus gland and optic ganglia

Animals		Sinus gland		Med. terminalis		3 distal ganglia	
Width	Sex	Right	Left	Right	Left	Right	Left
16.5 mm.	♀	93	97.5	1020	1070	1120	1610
17 mm.	♀	109	51	1320	1290	1690	1630
17 mm.	♂	34	26.5	1790	1300	1980	1700
19 mm.	♂	75	79	1650	1480	1650	lost
Total weights		565		10,920		11,380×8/7=13,006	
Ratio		1		19.3		23	

these small, soft organs, and the necessity of using sufficient numbers of organs in an extract to ensure even approximate consistency in results.

There are two sources of error which contribute to making the relative size of the sinus gland appear larger than is actually the case:

(1) While the ganglia could be picked free of adherent tissue, some extra tissue generally was included with the tiny sinus glands.
(2) The small size of the sinus gland allowed it to rest in the 0.1 mm. space between haemocytometer slide and cover glass without appreciable flattening. The projection of its outline thus encompassed some tissue actually less than 0.1 mm. in thickness.

These sources of error may make the sinus gland appear perhaps nearly twice its true size, relative to the optic ganglia. For purposes of dilution, however, it was assumed to have a bulk of $\frac{1}{20}$ that of the medulla terminalis and of the group of distal ganglia.

D. *Estimation of degree of dark-adaptation*

This was done by noting the extent of "glow," or light-reflecting area in the eye. Animals to be examined were picked up in dim red light and their eyes examined with the aid of a 6-volt "Mignon" lamp held close to the observer's line of vision, an operation requiring only a few seconds. Because of the irregular shape of the crab "cornea," it is difficult to make a quantitative estimate of the amount of "glow" present, especially since the "glow" is more pronounced and more often seen in the most ventral portion of the retina than elsewhere. The following scheme of expressing the degree of dark-adaptation in retinal pigments was followed, with the realization that the four stages and one doubtful intermediate stage (±) indicated are not necessarily proportional to the activity of the photo-adaptive mechanism:

Symbol	Description
0	Fully light-adapted. No glow visible.
±	Indistinct glow in ventral region of one or both eyes.
+	Glow distinct, but restricted to ventral region of eye.
++	Glow distinct in ventral region, with sub-maximal glow visible in long axis of eyestalk.
+++	Fully dark-adapted. Pronounced glow visible in long axis of eyestalk.

The use of "+" symbols was convenient in recording retinal pigment positions in those cases in which the state of body chromatophores (indicated by numbers 1–5) was studied in the same animals. The diurnal pattern of retinal pigment migrations was plotted with respect to time, the resulting curve serving to show the general trend of events. Since only three or four observations were made per day in order to avoid excessive stimulation by handling and bright light, the curves (whose exact form is of little importance in the present study) are a somewhat stylized expression of the day-to-day pattern of retinal pigment migration.

E. *Sinus gland extirpation and control operations*

Experimental animals were operated upon by means of a small dental excavating burr mounted in a jeweler's screwdriver handle, permitting the drill to be rotated

with the fingers of one hand. With the crab securely wrapped in wet cloth and held under the medium power of a dissecting microscope, a hole somewhat less than 1 mm. in diameter was drilled through the dorsal side of the eyestalk. The pigmented hypodermis was then slit and laid aside with a needle, revealing (if the hole were correctly located) the sinus gland, easily recognized by its glistening bluish-white appearance against the gray of the optic ganglia. The upwelling of blood usually raised the gland so that it could be teased free with a needle or sharp-pointed watchmaker's forceps and removed. In our experience the removal of sinus glands from these crabs is easier than from the crayfish, because of the superficial location and more compact form of the gland. The wound was cauterized superficially to coagulate the blood across the opening to reduce bleeding. It was found best to wait several hours after the first operation before removing the second sinus gland. In about 10 per cent of cases the gland was broken in removal, necessitating considerable probing to retrieve all portions of it, and causing much bleeding. In cases where the opening was cut at a point not over the gland, it proved best not to attempt to seek out the gland, but to use the operation as a control. In control operations the ganglia proximal to the sinus gland were in some cases slashed with a needle point in the effort to sever the nerve supplying the gland without cutting the optic tracts; in other cases the entire optic tract was severed. The extent of injury inflicted in sinus gland removal on small crabs proved to be difficult to evaluate but was less than we have experienced in carrying out sinus gland removal in crayfish following the method of Brown (1942). The sinus gland could generally be picked from its superficial position with no apparent damage to nerves and good healing took place, yet serial sections generally revealed some degree of nerve injury, often severe enough to make it impossible to attribute the observed results simply to the absence of the sinus gland. Eyestalks were routinely fixed and sectioned serially after observations, but because of the number and the refractoriness of the heavy-walled crab eyestalks, the results of sectioning left much to be desired.

Mortality from operating was not heavy, except in the case of animals still soft from a recent moult, or those which moulted within a few days after an operation. Among animals not so weakened, deaths from bilateral sinus gland removal have amounted to 11 per cent and from operations involving nerve sectioning to 19 per cent, in some 35 and 25 animals respectively, operated upon in the series of experiments reported here. The writer recognizes that the method of sinus gland removal employed upon these small crabs does not permit the control of bleeding and minimization of nerve injury attainable with the more precise methods which Kleinholz (1947) has used upon crayfish and lobsters. It was felt, however, that results obtained on large numbers of small, responsive, easily kept animals would aid in the definition of problems to be attacked later in larger crabs kept under better conditions than have been available locally.

Observations

A. *The normal rhythm of retinal pigment migration*

Normal crabs kept in the laboratory showed the characteristic changes in the position of the retinal pigments in response to the daily alternation of light and darkness, exhibiting a well-marked glowing "pseudopupil" in the eyes at night and the absence of such during daylight hours. This migration stopped under continuous

illumination (tested in a windowless room illuminated by two 150-watt bulbs in globes 7 feet above the animals), and the crabs remained in a continuously light-adapted state, not showing the diurnal rhythm of retinal pigment activity reported for certain other crustaceans. On the other hand, crabs kept in continuous darkness exhibited the typical diurnal rhythm of retinal pigment migration, becoming fully dark-adapted during the night hours and fully light-adapted during the day.

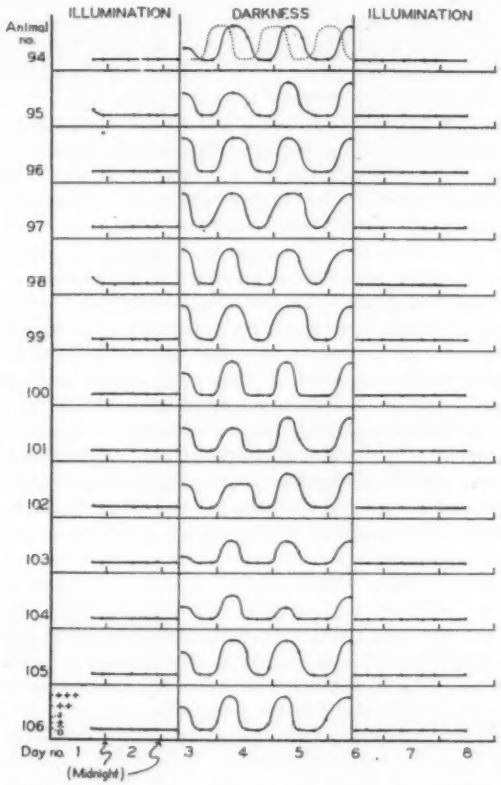


FIGURE 1. Diurnal migrations of retinal pigments exhibited in darkness but absent under illumination. Rhythm in darkness put out of phase with solar day by starting dark-adaptation at dawn. Dotted curve represents a "normal" rhythm.

This activity continued undiminished for at least 10 days, although occasional animals gradually became "out of phase" with the solar day. Ordinarily night-adaptation commenced in the late afternoon, even before sunset, while the reverse process of day-adaptation was often under way before dawn. The most striking case of a shift in phase of the diurnal activity cycle occurred when a group of *Hemigrapsus oregonensis* which had been exposed to continuous light for 40 hours were

transferred to the darkroom at 7 A.M. They soon became dark-adapted, and thereafter exhibited a period of dark-adaptation starting after midnight and lasting until early afternoon—an example of the reality of a diurnal activity rhythm as well as of its lability (Fig. 1).

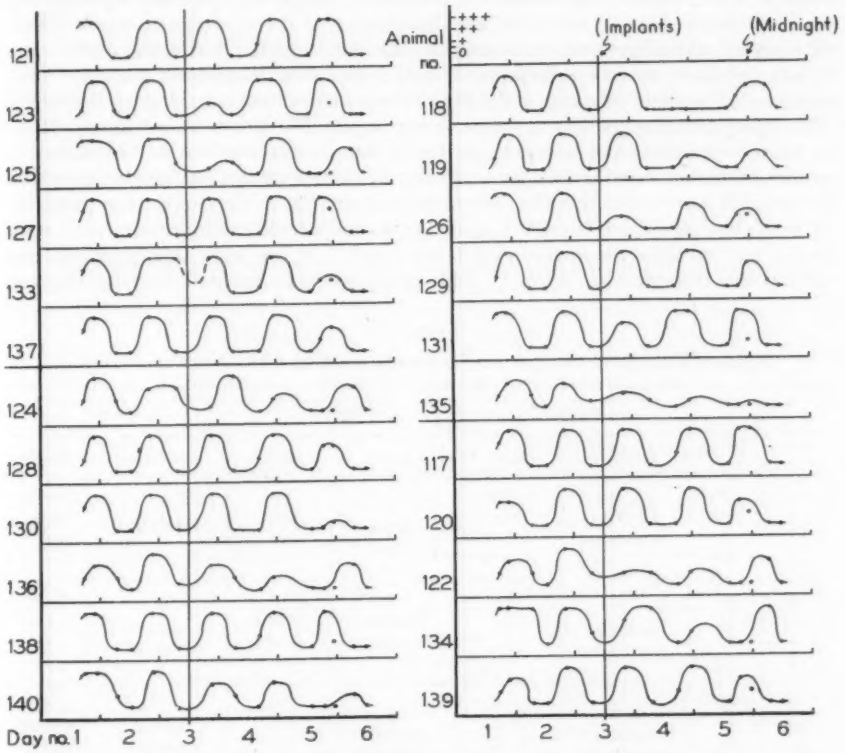


FIGURE 2. Relative ineffectiveness of a sinus gland or other tissue (implanted on third day) in suppressing diurnal rhythm of retinal pigments of crabs kept in darkness. 121–137, one sinus gland; 124–140, one medulla terminalis; 118–135, one group of three distal optic ganglia; 117–139, muscle. Note that on the fifth night too close spacing (95 minutes) between observations decreased the degree of dark-adaptation obtained.

B. *The effects of injections of sinus gland and nervous tissue extracts*

Over 40 tests were made in which sinus glands or optic ganglia were implanted by the technique of Scudamore (1947, p. 190) into the bases of walking legs of crabs the same size as the donors. The recipient crabs were observed in constant darkness to determine if the implanted tissues would suppress the normal diurnal cycle of the retinal pigments. While there was some evidence that a factor active upon retinal pigments was present in nervous tissues as well as in sinus glands, the results were not conclusive, as shown in Figure 2, which is typical of the whole series. On

the other hand, extracts of whole eyestalks injected into night-adapted animals of all three species in darkness caused a migration of retinal pigments to the "day" position, as evidenced by a complete or nearly complete disappearance of the "glow." Since it had early been observed that if crabs were inspected for "glow" in the darkroom at night, there often occurred a transitory change to a partially light-adapted state within the hour or two after the illumination of the eyes (as shown in Fig. 2 fifth night), and since these crabs only became dark-adapted during the night hours it was necessary to time injections in such a way that all animals could be safely assumed to be night-adapted at the time of injection without making an inspection. The procedure adopted was as follows: a group of 24 crabs were measured, placed in numbered dishes, and brought into the darkroom at about 6 P.M. Inspection at a later hour that evening and again during the following day sufficed to show that the animals were exhibiting the normal diurnal rhythm. They were then presumed to be night-adapted by 9 or 10 P.M. of the second evening. Injections were made under dim red light, the time of each being noted. At one hour after injection, each animal was inspected for "glow." This procedure was repeated the next two eve-

TABLE II

Effects of sinus gland and optic ganglia extracts, tested upon Hemigrapsus oregonensis

Crab no.	Width (mm.)	March 7		March 8		March 9	
		Injection	Result	Injection	Result	Injection	Result
1	21	Control injection	+++	½ sinus gland	+	Muscle	+++
2	19		++		0		++
3	17		+++		±		+++
4	19		+++		+		+++
5	20	½ medulla terminalis	+	Control injection	+++	½ sinus gland	Died
6	18		+		+++		0
7	17		+		+++		+
8	20		+		+++		±
9	19	1/40 medulla terminalis	++	½ medulla terminalis	+	Control injection	+++
10	17		++		+		+++
11	21		++		+		+++
12	18		++		+		+
13	20	½ distal ganglia	+	1/40 distal ganglia	+++	½ medulla terminalis	±
14	19		+		++		±
15	16		±		+++		±
16	18		+		+++		+
17	21	Muscle	+++	½ distal ganglia	+	1/40 sinus gland	+
18	18		+++		+		++
19	17		+++		±		+
20	18		+++		+		++
21	17	½ sinus gland	+	Muscle	+++	½ distal ganglia	0
22	18		+		+++		+
23	17		±		++		±
24	17		0		+++		0

nings, so that injections were given to the same group of crabs for three successive nights. Since the injections often interrupted the normal pattern of diurnal migration of pigments, it was surprising that consistent results could be obtained even for three nights. After such a series of injections, the test animals were discarded, since the tendency to get out of phase with the solar day introduced an element of uncertainty.

There appear, in Tables II and III, the results of two such series of injections of extracts of sinus gland, nervous, and muscular tissues. Extracts were from light-adapted crabs of the same species and of the same average size as the recipients, and all injections were of 0.05 cc. of extract, the concentrations being expressed as the fraction of an "average" organ injected. A given extract was used for one series of three successive injections. Control injections were of crab perfusion fluid.

TABLE III

Effects of extracts of brain, thoracic nerve mass, optic ganglia and sinus gland, tested upon Hemigrapsus oregonensis

Crab no.	Width (mm.)	March 14		March 15		March 16	
		Injection	Result	Injection	Result	Injection	Result
25	18	Brain ½ hemisphere	+	Brain 1/40 hemisphere	++	1/10 thoracic nerve mass	+
26	20		++		+++		±
27	19		+		+		Died
28	21		+		++		+
29	17	1/10 thoracic nerve mass	++	1/200 thoracic nerve mass	++	½ medulla terminalis	±
30	18		++		+++		±
31	17		++		++		±
32	20		++		++		+
33	18	½ medulla terminalis	±	1/40 medulla terminalis	++	Muscle	+++
34	18		±		++		++
35	18		+		++		+++
36	19		++		++		+++
37	18	Muscle	+++	Muscle	+++	½ sinus gland	0
38	18		+++		+++		±
39	18		+++		+++		0
40	21		+++		+++		±
41	17	½ sinus gland	+	1/40 sinus gland	+	Control injection	+++
42	21		+		+		+++
43	16		±		+		+++
44	17		±		++		++
45	17	Control injection	+++	Control injection	+++	Brain ½ hemisphere	+
46	20		+++		++		±
47	17		+++		+++		Died
48	20		+++		+++		+

While these injection methods are only roughly quantitative, several generalizations may be drawn from the results:

(1) The total retinal pigment activating potency of one sinus gland is roughly equivalent to that found in one medulla terminalis, or in one group of the three more distal optic ganglia.

(2) Since the bulk of either of these ganglionic masses is at least 20 times that of the sinus gland, it would appear that the active principle is at least 20 times more concentrated in the latter organ.

(3) Of the total active substance extractable from the eyestalk, roughly ⅓ resides in the sinus gland. This is in contrast to the distribution of body-chromatophore-activator in the eyestalks of several species of shrimps and crabs studied by Brown (1940) in which at least 80 per cent of the total activity resides in the sinus gland.

(4) Nervous tissue other than optic ganglia also contains a retinal pigment activating principle, in a concentration of the same order of magnitude as is found in optic ganglia.

The above facts suggest that the production of retinal pigment activator is not restricted to the sinus gland, but occurs in other parts, or perhaps all parts, of the nervous system. The active substance may, however, be more efficiently produced or quantitatively stored in the sinus gland, which in turn might be specialized for its periodic release. Upon this basis, attempts were made to block the diurnal retinal pigment changes by operative removal of sinus glands.

C. *Sinus gland removal in animals exposed to normal darkness and daylight*

Bilateral sinus gland removal was carried out upon a group of 13 crabs and the animals observed daily and nightly for periods of 3–5 days. The presence or absence of "glow" was noted but sufficient data to show the time course of pigment migration were not collected. The results of sinus gland removal, as observed in the living animals, were quite variable, but in no case did a glow appear during daylight as the result of the operation. A glow was generally present at night, but was variable in amount, ranging from a practically normal glow down to a slight or questionable glow. Both eyes of each of these animals were fixed and sectioned serially, one eye being removed in bright daylight, and the other at night, under dim red light. A summary of the observations with the results of a study of serial sections of the eyestalks follows (Table IV).

As a control operation, the optic tract was severed within the eyestalks of 8 animals by the severe procedure of stabbing the region of the medulla terminalis or interna with a heated needle, after drilling a hole as for sinus gland removal. The sinus gland itself was not touched. With two exceptions the animals in this group failed to show night adaptation. Histological examination revealed that in at least three animals, possibly because of damage to blood supply, the retina and distal optic ganglia were in a necrotic state. In such animals the apparent state of light-adaptation is perhaps comparable to that reported by Bennitt (1924) as due to death or to separation of the eyestalk from the body rather than to processes related to denervation or interference with sinus gland function. The results obtained on the remaining five animals are summarized in Table V.

The effect of optic nerve severance seems clearly to be a continued state of day-adaptation, a result consistent with the view advanced by Welsh (1941) that the light-adapted state is brought about by the release of a sinus gland hormone when a tonic nervous inhibition of the gland is reduced. Yet the view that a sinus gland

TABLE IV

Results of histological examination; sinus gland removal; normal daylight and darkness

Record number	Species	Response	Sinus glands	Nerve damage
9	*H. oregonensis*	Normal	Complete removal	Slight
11	*H. oregonensis*	Right: normal Left: impaired night adaptation	Complete removal	Right: slight Left: considerable
13	*H. oregonensis*	Impaired night condition	Complete removal	Right: optic tract severed Left: some damage
16	*H. oregonensis*	Right: intermediate Left: normal	Complete removal	Right: necrotic Left: slight damage
18	*P. crassipes*	Normal	Complete removal	Slight
21	*P. crassipes*	Impaired night adaptation	Right: removed Left: some remaining	Right: slight Left: severe
24	*H. nudus*	Normal	Complete removal	Slight
39	*H. nudus*	Impaired night adaptation	Complete removal	Right: severe Left: considerable
40	*H. nudus*	Normal	Complete removal	Slight
46	*H. nudus*	Impaired night adaptation	Complete removal	Moderate to severe
47	*H. nudus*	Right: normal Left: impaired night adaptation	Right: some remaining Left: removed	Right: slight Left: optic tract severed (?)
49	*H. nudus*	Impaired night adaptation	Right: trace remaining Left: removed	Slight
50	*H. nudus*	Impaired night adaptation	Right: trace remaining Left: removed	Slight

hormone is solely responsible for inducing the movement of pigment to the "day" position is not supported by the results of sinus gland removal, which in no case caused the eye to become permanently "dark-adapted;" in fact any changes that were produced were in the direction of increased day-adaptation, a result possibly attributable to nerve damage incidental to sinus gland removal. Furthermore, the possibility exists that, in the absence of sinus gland hormone, the retinal pigments might possess some power to act as independent effectors in a direct response to light. Accordingly other series of observations were made under conditions of continuous darkness.

TABLE V

Results of histological examination; optic nerve sectioning; normal daylight and darkness

Record number	Species	Response	Sinus glands	Nerve damage
12	*H. oregonensis*	Permanent "day" adaptation	Damaged (?)	Optic tracts severed
25	*P. crassipes*	Permanent "day" adaptation	Intact	Optic tracts severed
41	*H. nudus*	Permanent "day" adaptation	Right: intact Left: damaged	Right: optic tract severely damaged Left: optic tract severed
42	*H. nudus*	Right: day adapted Left: nearly normal	Intact	Right: optic tract severed Left: optic tract severely damaged
45	*H. nudus*	Right: day adapted Left: impaired night adaptation	Intact	Right: optic tract severed Left: optic tract damaged

D. *Sinus gland removal in animals exposed to continuous darkness*

The effects of sinus gland removal and optic tract damage were tested upon a group of 25 animals of all three species, both males and females, selected to include no soft, recently moulted crabs. In the course of the experiment, five moulted and did not survive. Observations were first made on the intact animals for three days and nights, to obtain a record of the pattern of the diurnal rhythm in each animal (see Fig. 3). On the fourth day the group was returned to a lighted room, fed, given fresh seawater, and received the first operation upon the right eyestalk. On the following day the second operation was performed on the left eyestalk, and the animals returned to darkness. Observations continued over the next five days and nights (with one night on which no check could be made). By reference to Figure 3 it may be seen that in the four normal control animals the rhythm continued unchanged.

The results of operations again showed considerable variation. In this series control operations were restricted to a superficial cut across the optic tracts proximal to the sinus glands, in the effort to denervate the gland without severing the main optic tracts. Of the six survivors, four showed no real change, while in two animals (nos. 57 and 63) one eye continued to show diurnal changes and the other did not. Histological examination of the eyes in this group seemed to indicate that in those two eyes which failed to show pigment migration, nerve damage was more severe than in those eyes which continued to show diurnal pigment migration, but the evidence was far from satisfactory. The fact that the two eyes of a single animal may show different degrees of adaptation has been noted often enough in this work to indicate a considerable degree of independent control of the pigments in each eye.

Ten animals survived bilateral sinus gland extirpation (of these, nos. 54, 65, and 76 had the left eyestalk removed, observations being carried out on the right

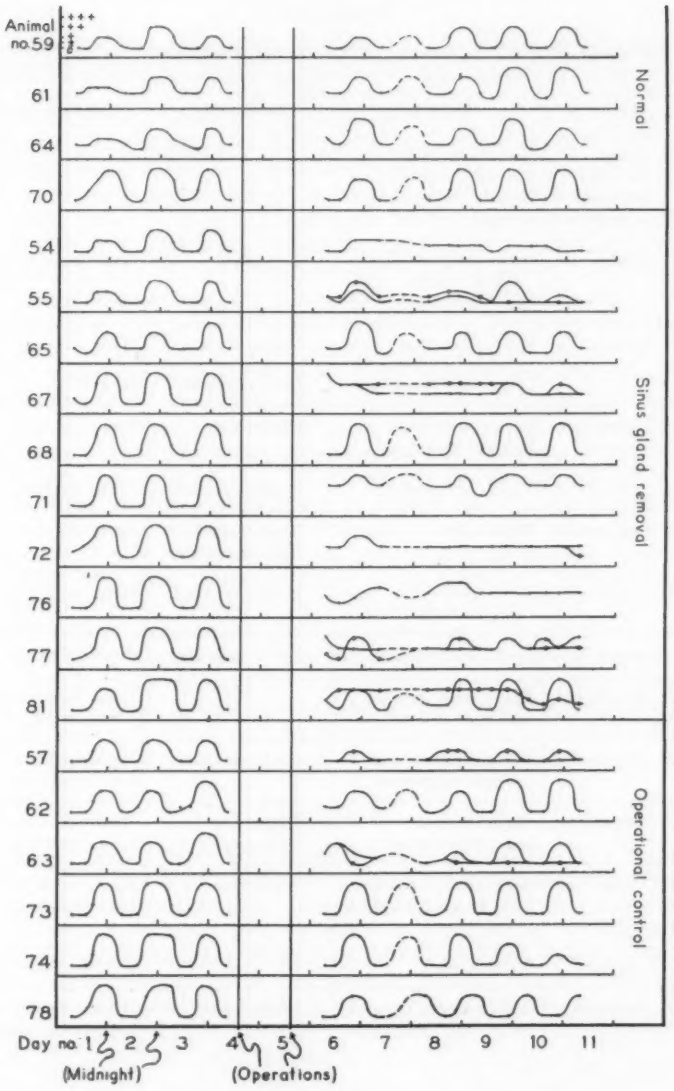


FIGURE 3. The effects of sinus gland removal and optic nerve damage upon the diurnal rhythm of retinal pigment migration of crabs kept in darkness. Double curves indicate a difference in the two eyes of one animal, the open circles indicating the left eye. Single curves indicate that both eyes were the same.

TABLE VI

Results of histological examination; sinus gland removal and control operations; continuous darkness

Record number	Species	Response	Sinus glands	Nerve damage
59	*H. nudus*	Normal (control)	—	—
61	*P. crassipes*	Normal (control)	—	—
64	*P. crassipes*	Normal (control)	—	—
70	*H. oregonensis*	Normal (control)	—	—
54	*H. nudus*	Right: approaching "day" state Left eye removed	Removed —	Optic tract severed —
55	*H. nudus*	Right: impaired "night" migrations Left: "day" condition	Right: trace Left: removed	Slight Severe
65	*P. crassipes*	Right: normal Left eye removed	Right: trace —	Slight —
67	*H. oregonensis*	Both: intermediate	Both: removed	Both: moderate
68	*H. oregonensis*	Both: normal	Right: trace Left: removed	Both: slight
71	*H. oregonensis*	Both: migrations, tending to "night" state	Both: removed	Both: moderate
72	*H. oregonensis*	Both: intermediate	Both: removed	Both: moderate
76	*H. oregonensis*	Right: intermediate Left eye removed	(?) —	(Lost in sectioning; no evaluation)
77	*H. oregonensis*	Both: intermediate, slight migrations	Both: removed	Right: severe Left: moderate
81	*H. oregonensis*	Right: normal Left: intermediate	Right: (?) Left: removed	Right: (lost in sectioning) Left: moderate
57	*H. nudus*	Right: "day" state Left: impaired night migrations	Both: intact	Right: optic tract severed Left: moderate
62	*P. crassipes*	Both: normal	—	(Not sectioned)
63	*P. crassipes*	Right: nearly normal Left: "day" condition	Both: intact	Right: slight Left: optic tract severed
73	*H. oregonensis*	Both: normal	Both: intact	Both: slight
74	*H. oregonensis*	Right: normal Left: (not operated upon)	Right: intact Left: (intact)	Right: slight Left: —
78	*H. oregonensis*	Both: normal	—	(Not sectioned)

eye only). The results were quite variable, nos. 54, 67, 72, and 76 exhibiting a loss of diurnal pigment movements; nos. 65, 68, and 71 continuing to show these changes; and nos. 55, 77, and 81 exhibiting diurnal rhythm in the pigments of one eye and not the other. Histological examination of the eyes in this group showed that there was great variation in the extensiveness of nerve damage in different cases. The following summary (Table VI) indicates the extent to which histological results (admittedly unsatisfactory) agree with the observed behavior.

The results shown in Figure 3 and Table VI bear out the earlier indications that severe damage to nerves can cause a persistent state of day-adaptation in the retinal pigments. The effects of sinus gland removal are less easy to evaluate. In the case of three animals (nos. 55, 65, and 68) the possibility of traces of the sinus gland remaining in the eyestalk cannot be eliminated. Even in cases where the gland itself is clearly absent, its nerve may be seen extending far into the medulla terminalis. Since the sinus gland nerve in life exhibits the same characteristic bluish appearance as the sinus gland itself, and shows in fixed material the presence of an eosinophilic secretion product, it must be admitted that it may well be capable of compensating to some extent for the loss of the gland proper. Perhaps of more significance are those animals (nos. 67, 71, 72, 76, 77, and the left eye of 81) in which sinus gland removal is followed by incomplete day-adaptation, and in which nerve damage, though present, is not excessive. Although sinus gland removal has failed, as before, to produce a full and persistent night-adapted state, it seems at least in these cases to have produced a shift in that direction. Since nerve damage tends to produce the day-adapted state, the intermediate condition may be the resultant of the effect of sinus gland removal promoting dark-adaptation and nerve damage causing light-adaptation. The fact that this "tendency" toward dark-adaptation is not wholly effective even in continuous darkness, does not in itself encourage one to assign an exclusive role in retinal pigment control to the sinus gland. Likewise the fact that the eyes of the same animal may behave differently is more easily attributable to unequal nerve damage than to sinus gland removal.

Discussion

The studies reported above were undertaken in an attempt to verify the generally accepted claim that day-adaptation in the decapod crustacean eye occurs under the influence of a hormone produced and released by the sinus glands. The work upon which this view is based has been done chiefly upon macrurans: shrimps and crayfish. The critical test of sinus gland removal still remains to be applied to the macrurans. Its application in the brachyurans, reported herein, while generally supporting previous contentions regarding the macrurans, does indicate that at least in the grapsoid crabs the sinus gland is not the sole source of retinal pigment activator. Despite numerous operations, simple sinus gland removal has usually failed either to cause the eye to remain dark-adapted or to cease its diurnal pigmentary changes, except that in continuous darkness there may be a lessened ability to assume the day-adapted condition during daylight hours. In all types of operations there has been shown a marked tendency for nerve damage to result in a more pronounced state of day-adaptation, a factor which should be taken into account when describing the effects of operations upon the sinus gland. The injection of extracts of other tissues indicates that while the sinus gland is clearly richer in active mate-

rial, nervous tissues contain large amounts, the sinus gland nerve showing visible secretion products like the sinus gland. If this material is utilized as a hormone, we are forced to consider the possibility that it can be released by nervous tissues in significant amounts, even in the virtual or complete absence of sinus glands.

Summary

1. The grapsoid crabs, *Hemigrapsus* and *Pachygrapsus*, exhibit in constant darkness a marked diurnal rhythm of retinal pigment migration.

2. This rhythm is absent in continuous light, and can be induced to become out of phase with solar day in constant darkness.

3. About ⅓ of the retinal pigment activator of the eyestalk resides in the sinus gland, with the remaining ⅔ distributed in the optic ganglia. Brain and other nervous tissues also contain an active principle in concentrations comparable to that in optic ganglia.

4. The sinus gland is at least 20 times richer than nervous tissue in retinal pigment activator.

5. Damage to nerves of the optic tract impairs the attainment of night-adaptation. If sufficiently severe, nerve damage may result in a state of permanent day-adaptation.

6. Operative sinus gland removal does not produce full dark-adaptation, a fact which may be in part explainable on the basis of concomitant nerve damage, but it does reduce the extent of day-adaptation in crabs kept in constant darkness.

7. It is concluded that, in the grapsoid crabs studied, the sinus glands are specialized (in addition to entirely different functions) for the elaboration and possibly the release of a principle active upon retinal pigments. On the present evidence it cannot be concluded that the sinus glands are the sole source of the retinal pigment activating hormone(s) in this group of crabs.

LITERATURE CITED

Bennitt, R., 1924. The migration of the retinal pigment in crustaceans. *J. Exp. Zool.*, **40**: 381–435.

Bennitt, R., 1932a. Physiological interrelationship in the eyes of decapod Crustacea. *Physiol. Zool.*, **5**: 49–64.

Bennitt, R., 1932b. Diurnal rhythm in the proximal pigment cells of the crayfish retina. *Physiol. Zool.*, **5**: 65–69.

Brown, F. A. Jr., 1940. The crustacean sinus gland and chromatophore activation. *Physiol. Zool.*, **13**: 343–355.

Brown, F. A. Jr., 1942. Sinus gland extirpation in the crayfish without eyestalk removal. *Proc. Soc. Exper. Biol. and Med.*, **50**: 295–297.

Brown, F. A. Jr., 1944. Hormones in the Crustacea: Their sources and activities. *Quart. Rev. Biol.*, **19**: 32–46 and 118–143.

Kleinholz, L. H., 1934. Eye-stalk hormone and the movements of the distal retinal pigment in Palaemonetes. *Proc. Nat. Acad. Sci.*, **20**: 659–661.

Kleinholz, L. H., 1936. Crustacean eye-stalk hormone and retinal pigment migration. *Biol. Bull.*, **70**: 159–184.

Kleinholz, L. H., 1942. Hormones in Crustacea. *Biol. Rev.*, **17**: 91–119.

Kleinholz, L. H., 1947. A method for removal of the sinus gland from the eyestalks of crustaceans. *Biol. Bull.*, **93**: 52–55.

PANOUSE, J. B., 1947. Les corrélations humorales chez les crustacés. *L'Année Biologique*, **23**: 33–70.

SCUDAMORE, H. H., 1947. The influence of the sinus glands upon molting and associated changes in the crayfish. *Physiol. Zool.*, **20**: 187–208.

WELSH, J. H., 1939. The action of eye-stalk extracts on retinal pigment migration in the crayfish, Cambarus bartoni. *Biol. Bull.*, **77**: 119–125.

WELSH, J. H., 1941. The sinus glands and 24-hour cycles of retinal pigment migration in the crayfish. *J. Exp. Zool.*, **86**: 35–49.

THE PROTHORACIC GLANDS OF LEUCOPHAEA MADERAE (ORTHOPTERA)

BERTA SCHARRER[1]

Department of Anatomy, University of Colorado Medical Center

Introduction

Of the insect organs which furnish developmental hormones the corpora cardiaca-allata and the neurosecretory centers of the brain have been known for some time to be operative in a number of orders. More recently the endocrine role of another organ of internal secretion, the prothoracic glands, has been demonstrated in *Lepidoptera*. In this group of insects an active principle produced by these glands is necessary for pupation and imaginal differentiation, and apparently also for molting (Fukuda, 1940, 1941; Williams, 1947). These observations suggest that the factor produced by the prothoracic glands should be classified as a growth and differentiation hormone (see Scharrer, in press).

In a recent publication Williams (1948) discussed the still meager histological data available at present in regard to the prothoracic glands of certain *Lepidoptera*. In the same paper he mentioned the existence of prothoracic glands in the larva of *Cimbex americana* (*Hymenoptera*), and suggested that the organs described as "ventral glands" in *Dixippus morosus* (*Orthoptera*) by Pflugfelder (1938, 1939) may be homologous to the prothoracic glands of the *Lepidoptera*. Aside from these data there is little morphological evidence of the occurrence of prothoracic glands in insects other than *Lepidoptera*.

Since the existing experimental evidence suggests an important role of the prothoracic glands, it appears desirable to study their occurrence among various groups of insects, their histology and appearance in different phases of the life cycle, and their relationship to other endocrine organs. The investigation reported here concerns the histology of the prothoracic glands of *Leucophaea maderae* (*Orthoptera*) in different stages of postembryonic development and their involution after metamorphosis.

Material and Methods

The observations are based on sectioned tissue (74 specimens) and on supravitally stained material (over 60 specimens). The animals used were various nymphal instars in different phases of the intermolt period, as well as male and female imagoes of *Leucophaea maderae* ranging from one hour to 15 months of "adult age." In the freshly opened insect the prothoracic glands are very difficult to see on account of their transparency which does not permit their differentiation from neighboring structures. The identification of the paired organ is greatly facili-

[1] The work was done while the author held a Fellowship from the John Simon Guggenheim Foundation. Support by grants from the American Cancer Society and The Anna Fuller Fund is gratefully acknowledged.

tated by the use of supravital dyes with which the prothoracic glands stain conspicuously. Specimens having received injections of neutral red or methylene blue solutions prior to dissection offer a suitable material for the study of certain morphological features of the gland. For permanent preparations the prothoracic region was fixed in Zenker-formol, Bouin, or Carothers solution. Paraffin sections 3–7 micra thick were stained with the following techniques: Hematoxylin-eosin, Mallory-azan, Masson's and Lendrum's trichrome, and Wilder's reticulum stains. Isolated glands were incubated at 56° C. in 2 per cent osmic acid solution for several hours and were studied in toto or after paraffin embedding.[2]

Observations

The prothoracic glands of *Leucophaea* consist of two bands of tissue which occupy a ventral position in the anterior thorax in close proximity to the prothoracic ganglion. The two bands cross in the manner indicated in Figure 1; at the point of

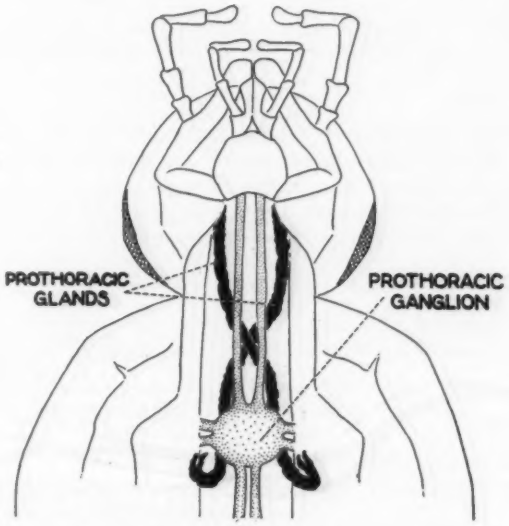


Figure 1. Ventral aspect of anterior thorax and head (tilted back) of *Leucophaea* nymph showing position of prothoracic glands.

crossing the organs are connected by a narrow tissue bridge. The anterior ends of the tissue bands taper off in the neck region. At the posterior end each band divides into two short branches one of which establishes nervous connection with the prothoracic ganglion. Figure 2, based on methylene blue preparations, shows a branch of a thin nerve entering the prothoracic gland shortly after its emergence from the lateral surface of the ganglion. No ducts are found in connection with

[2] I am indebted to Miss Kate Gruen for valuable technical assistance.

these glands; they are surrounded by blood spaces. It may be assumed, therefore, that the secretory products are given off into the blood.

The prothoracic glands are present in both nymphs and adults, but in the latter they become considerably reduced in size and structure soon after emergence.

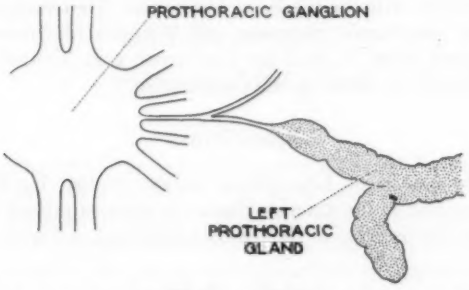


FIGURE 2. Innervation of prothoracic gland of *Leucophaea* by a lateral branch from the prothoracic ganglion, as shown for left side. Diagram based on methylene blue preparations.

A. The nymphal type

In the prothoracic gland of the nymph and of the freshly emerged adult the cells are arranged in densely packed layers around the longitudinal axis of the tissue band. The center is occupied by a trachea, a nerve, and several parallel fibers of striated muscle all of which extend throughout the length of the organ (Figs. 3, 4). The nerve is evidently derived from the prothoracic ganglion, since no other nervous

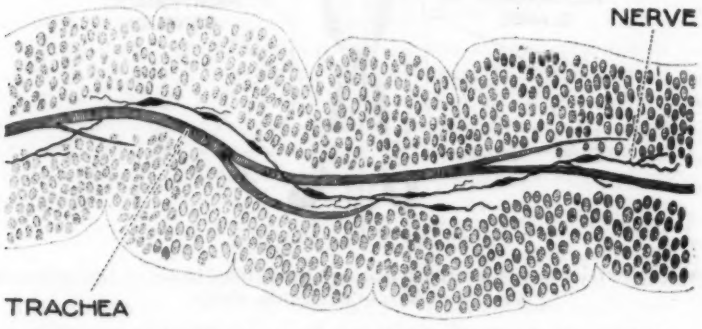


FIGURE 3. Prothoracic gland of nymph of *Leucophaea* stained supravitally with methylene blue.

branch than the one mentioned above (Fig. 2) has been observed to enter the gland. The central nerve, in addition to innervating the musculature of the organ, probably also supplies the glandular tissue; the delicate branches of the nerve could be traced only for short distances in methylene blue preparations. Due to the contraction of the axial muscle the glandular tissue in fresh and in fixed preparations is more or

less folded, with the result that the width of the organ varies. In the wider portions 8 to 12 nuclei may be counted across the width of the cellular layer of one side (Fig. 3).

The deeply staining nuclei are the most prominent feature of the gland. They are ovoid and, for the most part, approximately uniform in size and appearance. In regard to their dense arrangement and general morphology they resemble the nuclei of the corpora allata of the same species. In some nymphal glands a number of considerably larger nuclei may be observed. The majority of specimens studied showed some pycnotic nuclei in the prothoracic glands.

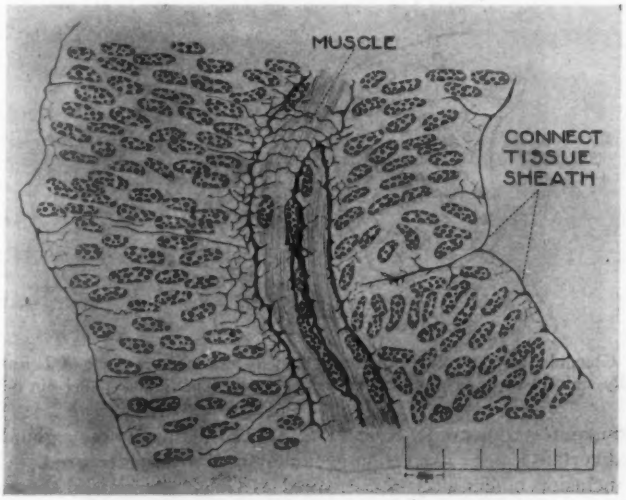


FIGURE 4. Prothoracic gland of last instar female nymph of *Leucophaea* in longitudinal section. Paraffin, 4 micra, Mallory azan.

The cytoplasm is not abundant and the cell boundaries are difficult to discern, except under favorable conditions as, for instance, in the periphery of the sectioned organ, where the nuclei are less densely packed.

With the methods employed for permanent preparations no appreciable amount of cytoplasmic inclusions indicating a secretory activity of the prothoracic gland cells was observed. In two specimens a few acidophilic granules were seen, in others the cytoplasm showed vacuolization. These observations suggest the possibility that certain cytoplasmic inclusions may be dissolved during the ordinary embedding and staining procedure. This view is further supported by the results of supravital staining. The cytoplasm of fresh tissue subjected to supravital dyes for a suitable period of time contains inclusions, as indicated in Figure 5. Round bodies varying in diameter stain a distinct blue with methylene blue. The staining is not always uniform throughout the particle; more deeply stained areas may be differentiated from a lighter background. The granular inclusions found to stain red with neutral

red are the same as those taking up methylene blue. This can be demonstrated by placing a methylene blue treated organ in a drop of weak neutral red solution on a slide. When a cover slip is used, the blue stain soon disappears from the granules and is replaced by a red tint. The same individual granules can be observed as they change color.

After incubation with osmic acid the cells of the prothoracic glands show blackened inclusions which, on account of their size and distribution, seem to be identical with the granules appearing in supravitally stained organs. These observations suggest that the granular inclusions are lipid in nature. Further than that no definite conclusions can be drawn. On the one hand, these bodies may be interpreted as

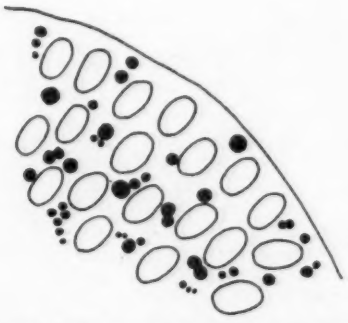


FIGURE 5. Glandular portion of prothoracic gland of last instar nymph of *Leucophaea*, supravitally stained, showing granular inclusions (solid black) and nuclei (in outline).

cytological manifestations of a secretory activity of the prothoracic glands, since it is known that certain types of secretion granules stain with neutral red. On the other hand the inclusion bodies may be identified as Golgi material on the basis of their similarity, both as to appearance and stainability, with the Golgi elements described in other invertebrate material (Worley, 1944).

The prothoracic glands are ensheathed by a thin connective tissue membrane from which tenuous branches may be seen to enter the glandular tissue. These branches continue into the fibrous network surrounding the muscle elements in the center (Figs. 4, 6). These relationships are best observed in azan preparations where the fibrous elements stain a bright blue.

The cells of the prothoracic glands undergo mitotic divisions. Not all specimens studied showed mitoses. In a group of 44 dated nymphs, ranging from instar four to eight, 4 micra serial sections in the horizontal plane through the prothoracic glands were checked for mitotic figures. Specimens fixed immediately after molting, or at intervals up to six days following a molt, had no mitoses; neither did nymphs preparing for a new molt as indicated by the separation of the old cuticle from the epidermis. However, a certain number of the prothoracic glands from animals fixed during the remainder of the intermolt period, showed mitotic figures. While some of these specimens had low values (approximately one mitosis in ten sections), others had an average of up to eight mitoses per section. Since the intervals between two molts in *Leucophaea* are subject to considerable variation,

no more precise conclusion can be drawn than that at some time during the intermolt period a probably short, but considerable spurt of mitotic activity takes place in the prothoracic glands.

B. Adult type

In the male and female imago of more than eight days of "adult age" the prothoracic glands are considerably changed in appearance (Fig. 7). The tissue bands have become thin and the microscopic examination shows that they consist almost exclusively of the muscular core. Most, if not all, of the glandular cells have dis-

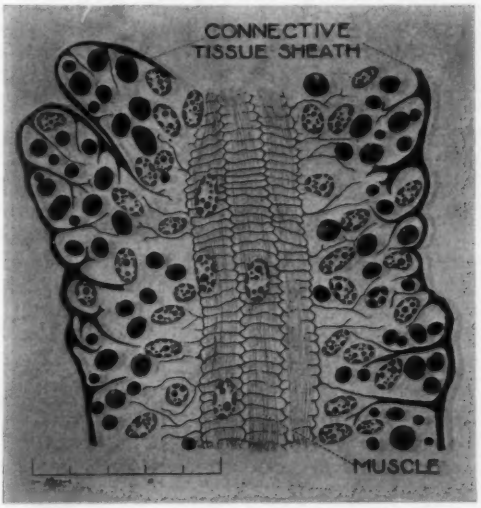


FIGURE 6. Longitudinal section of prothoracic gland of male adult *Leucophaea* four days after emergence, showing involutionary process (nuclear breakdown, decrease in width of glandular component). Paraffin, 4 micra, Mallory azan.

appeared. The connective tissue elements are more conspicuous than in the nymphal gland. This change of morphological appearance of the prothoracic glands within the short period of transition from the nymphal to the adult life may be interpreted as an indication that in the imago these organs are no longer functionally active. The steps of this striking involutionary process were traced in a series of adult male and female specimens, fixed at daily intervals after emergence. While the prothoracic glands of the freshly emerged adult resemble the nymphal organ, the first signs of regression may be noticed after 24 hours of adult life. At this stage a small number of the nuclei of the glandular component are pycnotic. Within the next three or four days the cellular breakdown becomes very conspicuous in that a large proportion of pycnotic nuclei or cellular remnants are interspersed with a gradually decreasing number of seemingly still normal nuclei (Fig. 6). The glandular tissue decreases in width, while no apparent change takes place with re-

spect to the muscular component. Animals fixed six, seven, or eight days after emergence show the involution of the prothoracic glands almost completed, practically all of the glandular tissue having disappeared by this time. From this stage on throughout adult life the organs show the same picture of involution (Fig. 7).

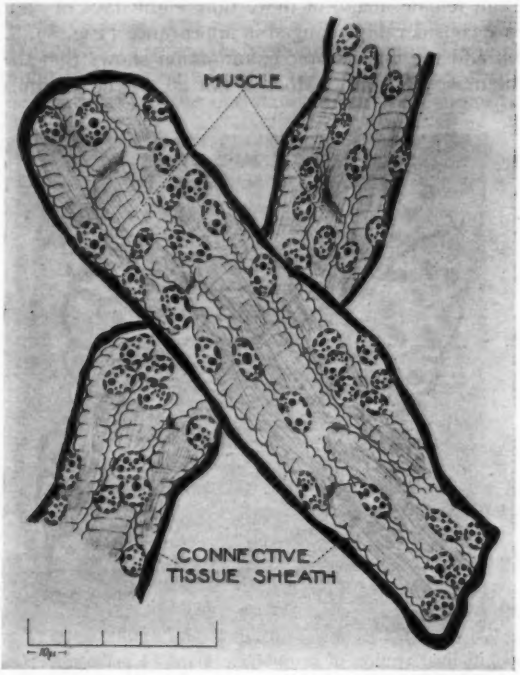


FIGURE 7. Longitudinal section (at point of crossing) of remnant of prothoracic glands of male adult *Leucophaea* one month after emergence. Note disappearance of glandular component, thickening of connective tissue sheath. Paraffin, 4 micra, Mallory azan.

DISCUSSION

The description given in the preceding paragraphs requires a few words of discussion of the place the organ in question occupies with reference to corresponding (homologous and analogous) structures. There can be little doubt that the prothoracic glands of *Leucophaea* are homologous with those of the *Lepidoptera*. Both are located in the anterior thorax. As in *Leucophaea* the prothoracic glands of certain *Lepidoptera* are more or less branched, band-like structures without ducts (Toyama, 1902/03; Lee, 1948). In both, the paired glands are composed of epithelioid cell elements with densely packed nuclei and a small amount of cytoplasm.

The innervation of the prothoracic glands does not represent a pertinent criterion for their identification, since a considerable variation seems to exist even within the *Lepidoptera*. In this group of insects the prothoracic glands may receive fibers

from the subesophageal ganglion, from the prothoracic ganglion, from the mesothoracic ganglion, and from the connectives between these ganglia (Lee, 1948). The glands of *Leucophaea* are innervated by fibers from the prothoracic ganglion, an observation which is not at variance with Lee's observations in *Lepidoptera.*

The prothoracic glands of *Lepidoptera* are morphologically well developed and are active as endocrine organs only in the immature insect; their presence has not been observed in the adult moth (Williams, 1948). In *Leucophaea* likewise the glandular component of the organ regresses after the emergence of the adult.

A feature characteristic of the prothoracic glands of *Leucophaea* is the presence of striated muscular tissue in the center of the organ. No such elements have been described in the corresponding organs of other insects with the possible exception of the "parenchymatous tracheal organs" of *Nepa* (p. 195). The physiological significance of this muscle is unknown. It is possible that it is instrumental in discharging the secretory product from the organ. This interpretation is suggested by the occurrence of muscular elements in exocrine glands, such as the Malpighian vessels of certain insects, likewise long, thin structures whose content is propelled towards the alimentary canal by a longitudinal muscle (Palm, 1946). Among vertebrates, contractile elements in the cytoplasm of the myoepithelial (basket) cells of certain glands (salivary, mammary, sweat, oral glands, etc.) also are thought to facilitate the expulsion of secretory products.

Another parallel exists in the occurrence of myoid cells (reticulum cells with striated fibrous elements) in the thymus of a number of vertebrates (see Bargmann, 1943). Their morphological similarity with the striated components of the prothoracic glands of *Leucophaea* is of interest in view of other features the prothoracic glands of insects and the vertebrate thymus appear to have in common (p. 195).

Whereas the homology between the prothoracic glands of *Leucophaea* and of *Lepidoptera* is reasonably well established, it is not always easy to recognize from the descriptions in the literature which of various organs in the head and thorax of different insects correspond to the prothoracic glands. Williams postulated that the "ventral glands" (Pflugfelder, 1938) of *Dixippus* (*Orthoptera*) are homologous with the prothoracic glands of *Lepidoptera.* In this connection it is highly interesting that Pflugfelder (1947) in a more recent publication extended his study of the ventral glands to include a number of insect orders, i.e., *Odonata, Ephemerida, Plecoptera, Saltatoria, Phasmida, Dermaptera, Blattaria, Mantodea,* and *Isoptera.* According to these data there exist in almost all lower *Pterygota* glandular organs of an endocrine character which, in their topography and histology, show many similarities. These phylogenetically ancient ventral glands are assumed to be derived from originally segmental organs whose function was excretory. In some cases the connection with the place of origin, i.e., the ventral epidermis, is still evident. More specifically, the ventral glands developed from the ectodermal canal of their precursor organs in the respective segment. During this transformation the lumen of the excretory duct gradually disappeared, a process which is indicated by the presence of a vestigial lumen in the ventral glands of *Plecoptera* and *Dermaptera.* The wall of the duct became the endocrine tissue.

It is of interest that a similar transformation from nephridial organs to glands of internal secretion occurred in certain crustaceans. The rudimentary antennal gland of the isopod *Asellus,* a serial homologue of the ventral glands of insects, is endocrine in nature (Pflugfelder, 1947).

Another recent publication must be considered in a discussion of the problem of homology of the prothoracic glands. Cazal (1947) described organs in *Aeschna* (*Odonata*) as "massifs ectodermiques intersegmentaires" which, at least with respect to their component situated in the posterior head region, seem to correspond to Pflugfelder's ventral glands. With the prothoracic glands of *Lepidoptera* and *Orthoptera* (*Leucophaea*), Cazal's organs have in common their occurrence in the thorax, their paired, elongated, irregularly lobated structure, and their histological appearance. Like the prothoracic glands of *Leucophaea*, the "intersegmental organs" of *Aeschna* consist of modified epithelial cells with scanty cytoplasm and closely packed nuclei and exhibit a typical cyclic behavior. A quiescent phase (after each molt) is followed by an active phase (preceding each molt), during which the nuclei show mitotic and pycnotic pictures. A "crisis" (crise cinétique) occurs in the adult which leads to the involution of the organ, comparable to that of the prothoracic glands of other insects, especially of *Leucophaea*.

An additional point of agreement exists between the observations in *Aeschna* and those reported in the present paper. The structure of the "intersegmental or-

TABLE I

	Antennal glands	1st maxillary glands	2nd maxillary glands	Thoracic nephridia		
				1	2	3
Crustaceans	Present, rudimentary, or absent (endocrine in Asellus)	Rudimentary, or absent	Present, rudimentary, or absent	Absent or present (Branchiura); without canal in Ostracoda	Absent or rudimentary (Ostracoda)	Absent or rudimentary (Ostracoda)
Onychophora	Rudimentary	Salivary glands	Present	Present	Present	Present
Diplopoda	Absent	Absent	Tubular glands	Absent	Absent	Absent
Chilopoda	Absent	Absent	Salivary glands?	Absent	Absent	Absent
Insects: *Apterygota*	Absent	Absent	Cephalic nephridia	Absent	Absent	Absent
"Lower" *Pterygota*	Absent	Corpora allata?	Ventral glands	Absent	Absent	Absent
"Higher" *Pterygota*	Absent	Corpora allata?	Salivary glands, spinning glands? prothoracic glands (Bombyx)	Prothoracic glands?	Absent	Absent

gans" according to Cazal is identical with that of the corpora allata of the same species. The striking similarity between these endocrine organs and the prothoracic glands in *Leucophaea* has been discussed (p. 189). Comparable observations in his material and a study of the literature led Pflugfelder (1947) to suggest that the corpora allata, like the ventral glands, may be derived from nephridial organs.

If we accept this derivation as correct, the ventral glands of the lower *Pterygota* (like the cephalic nephridia of the *Apterygota* and the second maxillary glands of crustaceans) can be considered as serial homologues of the corpora allata of pterygote insects, as well as of the antennal glands of crustaceans. No derivatives of thoracic nephridial organs are known to exist in the insect orders possessing ventral glands. In all holometabolous insects, in *Hemipteroidea*, in *Mantis*, and perhaps in *Blatta*, organs corresponding to the ventral glands of other insects studied by Pflugfelder are said to be absent. However, in some of these forms (*Lepidoptera, Hymenoptera, Blattaria*) organs situated in the thorax, the prothoracic glands ("hypostigmatic glands" of Toyama, "intersegmental organs" of Cazal) appear to take the place of the ventral glands. Furthermore, it is possible that a reinvestigation of the enigmatic "parenchymatous tracheal organs" in the thorax of the *Hemipteran Nepa* (Hamilton, 1931) may link these organs with the prothoracic glands of other insects.

The possible homologies of the prothoracic glands are summarized in Table I, which is based largely on Pflugfelder's data concerning the developmental history of nephridial derivatives in the *Articulata*.

With respect to *Bombyx*, Toyama (1902/03) showed that in the embryo the prothoracic glands develop from the second maxillary segment and, due to a shortening of this segment in the course of development, subsequently become located in the anterior thoracic region. If a corresponding derivation can be demonstrated in other forms possessing prothoracic glands these glands can be considered homologous with the ventral glands. However, it is quite possible that in certain insects the prothoracic glands may be shown to be derivatives of nephridia of the first thoracic segment, in which case they would be serial homologues of the ventral glands. The variability in the innervation of the prothoracic glands which may be supplied from ganglia of several segments (Lee, 1948; see also Pflugfelder, 1947) would thus be better understood.

The striking similarities in the development, structure, and life history of the ventral glands, the intersegmental organs, and the prothoracic glands suggest a correspondence in function (see Table II). It seems justified, therefore, to consider them as homologous organs whose endocrine function appears to be concerned with the control of developmental processes, both embryonic and postembryonic.

It has been mentioned before (p. 193) that the prothoracic glands of *Leucophaea* and their homologues also have some features in common with the thymus of the vertebrates; they are listed in Table III. The most interesting of these parallelisms are the involutionary process at the onset of sexual maturity, and the hastening of this regression by allatectomy [3] and hypophysectomy respectively.

The significance of such comparable traits is admittedly conjectural. However, it may be pointed out that there exist other structural and functional correlations between certain organs and organ systems of invertebrates and vertebrates, which

[3] These observations will be reported in greater detail in a later publication.

TABLE II

	Occurrence	Location	Morphology	Histology	Life history	Histological cycles relative to molting	Function
Ventral glands	Lower *Pterygota* (Pflugfelder '38, '39, '40, '47)	Ventrocaudal head region	Paired, elongate, lobated, originally with duct	Epithelioid cells of ectodermal origin, occasional very large cells (Saltatoria); structure similar to that of corpora allata	Presence in immature stages (embryonic and larval); regression in adults: nuclear fragmentation and degeneration (pycnosis); no regression in termite workers and soldiers	Secretory granules in intercellular spaces; mitoses before (last) molt and nuclear fragmentation	Assumed endocrine (regulation of development); dependency on corpora allata
Intersegmental organs (massifs ectodermiques intersegmentaires)	*Odonata: Aeschna* (Cazal '47)	Posterior head, thorax	Paired, elongate, lobated; intersegmental invagination continuous with epidermis	Epithelioid cells, dense nuclei, little cytoplasm; structure identical with that of corpora allata	Presence in immature stages, regression in adults ("crise cinétique")	Mitoses and pycnoses preceding molts, quiescent period following molts	Assumed control of nucleoprotein metabolism, comparable to lymphoid elements (thymus)
Prothoracic glands (hypostigmatic glands, Toyama)	*Lepidoptera* (Toyama '02/03, Ke '30, Fukuda '40, '41, Williams '47, '48, Lee '48)	Prothorax, mesothorax	Paired, elongate, lobated or branched, without duct	Large epithelioid cells, little cytoplasm	Presence in embryos, larvae and pupae, absence in adults		Endocrine: source of growth and differentiation hormone(s) regulating molting, pupation, and adult differentiation
	Hymenoptera: Cimbex (Williams '48)	Prothorax			Presence in larvae		
	Orthoptera: Leucophaea	Prothorax	Paired, elongate, without duct; good nervous and tracheal supply; muscular component	Epithelioid cells, little cytoplasm, occasional large cells; structure like that of corpora allata	Presence in nymphs, regression in adults	Cyclic mitotic activity	Assumed endocrine; dependency on corpora allata
Parenchymatous tracheal organs (?)	*Hemiptera: Nepa* (Hamilton '31)	Thorax	Muscular nature, rich tracheal supply		Regression in adults		Unknown

TABLE III

Prothoracic glands (and equivalents)	Thymus
Location in posterior head and thorax	Location in neck and thorax
Derived from bilateral invaginations of ectoderm	Derived from bilateral epithelial ingrowths
Ducts originally present, later rudimentary or absent	Ducts reduced to rudiments during development
Segmental development	Segmental derivation (from several pharyngeal pouches)
Syncytial structure (*Platysamia*)	Syncytial reticulum
Lymphocyte-like cell components (*Bombyx*)	So-called thymocytes of lymphocyte type
Muscular components (*Leucophaea*)	Myoid cells
Maximum size before emergence of adult, involution in imago	Maximum relative size before puberty, involution after puberty (higher vertebrates)
Influenced by endocrine disturbances (involution hastened by allatectomy, *Leucophaea*)	Sensitive toward endocrine imbalance, for instance hypophysectomy
Endocrine function established in *Lepidoptera* (control of developmental processes)	Doubtful endocrine function related to growth and development

cannot be dismissed as superficial and accidental similarities, but which indicate similar principles of functional organization. Thus the intercerebralis-cardiacum-allatum system of insects is analogous to the hypothalamo-hypophyseal system of vertebrates (Hanström, 1941; Scharrer and Scharrer, 1944), the internephridial organs of the worm *Physcosoma* to the interrenal body (Harms, 1921), the x-organ of crustaceans, to the thyroid, and the sinus gland of crustaceans to the paraphysis (Hanström, 1941). From these examples as from many others the concept evolves ever more clearly that the gap between invertebrates and vertebrates has in the past been magnified out of its true proportions.

SUMMARY

1. The prothoracic glands of *Leucophaea maderae* are paired band-like structures located in close proximity to and innervated by the prothoracic ganglion. The longitudinal axis contains striated musculature, a nerve and a trachea. The glands are well developed in nymphal stages, but regress in the imago.

2. In the nymph the glandular tissue consists of layers of dense nuclei surrounded by scanty cytoplasm. In their histological appearance the prothoracic glands strikingly resemble the corpora allata of the same species. The nymphal prothoracic glands exhibit a cyclic nuclear activity, characterized by a spurt of mitotic divisions during the intermolt period and by quiescent phases preceding and following each molt.

3. In freshly emerged male and female adults the prothoracic glands are still nymphal in appearance. Involution takes place during the first week of the adult stage. It manifests itself by a breakdown of nuclei and results in a reduction of the tissue bands to practically nothing except the muscular core.

4. The prothoracic glands of *Leucophaea* are considered to be homologous with the prothoracic glands of *Lepidoptera* and *Hymenoptera*, with the "ventral glands" of lower *Pterygota*, with the "intersegmental organs" of *Odonata*, and possibly with the "parenchymatous tracheal organs" of *Hemiptera*. They have certain features in common with the thymus of the vertebrates.

LITERATURE CITED

BARGMANN, W., 1943. Der Thymus. *Handb. mikr. Anat. Mensch.*, edited by W. v. Moellendorff, **6**: part 4, pp. 1–172.

CAZAL, P., 1947. Recherches sur les glandes endocrines retrocérébrales des insectes. II. Odonates. *Arch. zool. expér. gén.*, **85**: 55–82.

FUKUDA, S., 1940a. Induction of pupation in silkworm by transplanting the prothoracic gland. *Proc. Imp. Acad.* Tokyo, **16**: 414–416.

FUKUDA, S., 1940b. Hormonal control of molting and pupation in the silkworm. *Proc. Imp. Acad.* Tokyo, **16**: 417–420.

FUKUDA, S., 1941. Role of the prothoracic gland in differentiation of the imaginal characters in the silkworm pupa. *Annot. Zool. Japon.*, **20**: 9–13.

HAMILTON, M. A., 1931. The morphology of the water-scorpion, Nepa cinerea Linn. (Rhynchota, Heteroptera). *Proc. Zool. Soc.* London, **1931**: 3 and 4, pp. 1067–1136.

HANSTRÖM, B., 1941. Einige Parallelen im Bau und in der Herkunft der inkretorischen Organe der Arthropoden und der Vertebraten. *Lunds Univ. Årsskr.* N. F. Avd. 2, **37**: no. 4, 1–19.

HARMS, W., 1921. Morphologische und kausalanalytische Untersuchungen ueber das Internephridialorgan von Physcosoma lanzarotae nov. spec. *Arch. Entwickl. Mech.*, **47**: 307–374.

KE, O., 1930. Morphological variation of the prothoracic gland in the domestic and the wild silkworms (In Japanese with English summary). *Bulteno Scienca Fakult. Terkult.* Kyushu Imp. Univ. Fukuoka, **4**: 12–21.

LEE, T. Y., 1948. A comparative morphological study of the prothoracic glandular bands of some lepidopterous larvae with special reference to their innervation. *Ann. Ent. Soc. Amer.*, **41** (in press).

PALM, N. B., 1946. Studies on the peristalsis of the malpighian tubes in insects. *Lunds Univ. Årsskr.* N. F. Avd. 2, **42**: no. 11, 1–39.

PFLUGFELDER, O., 1938. Weitere experimentelle Untersuchungen ueber die Funktion der Corpora allata von Dixippus morosus Br. *Zeit. wiss. Zool.*, **151**: 149–191.

PFLUGFELDER, O., 1939. Wechselwirkungen von Druesen innerer Sekretion bei Dixippus morosus Br. *Zeit. wiss. Zool.*, **152**: 384–408.

PFLUGFELDER, O., 1947. Ueber die Ventraldruesen und einige andere inkretorische Organe des Insektenkopfes. *Biol. Zentralbl.*, **66**: 211–235.

SCHARRER, B. Hormones in insects. *Hormones, chemistry, physiology, and clinical applications*, edited by G. Pincus and K. V. Thimann, vol. I (in press).

SCHARRER, B., AND E. SCHARRER, 1944. Neurosecretion VI. A comparison between the intercerebralis-cardiacum-allatum system of the insects and the hypothalamo-hypophyseal system of the vertebrates. *Biol. Bull.*, **87**: 242–251.

TOYAMA, K., 1902/03. Contributions to the study of silk-worms. I. On the embryology of the silk-worm. *Bull. Coll. Agric.* Tokyo Imp. Univ., **5**: 73–118.

WILLIAMS, C. M., 1947. Physiology of insect diapause. II. Interaction between the pupal brain and prothoracic glands in the metamorphosis of the giant silkworm, Platysamia cecropia. *Biol. Bull.*, **93**: 89–98.

WILLIAMS, C. M., 1948. Physiology of insect diapause. III. The prothoracic glands in the Cecropia silkworm, with special reference to their significance in embryonic and post-embryonic development. *Biol. Bull.*, **94**: 60–65.

WORLEY, L. G., 1944. Studies of the vitally stained Golgi apparatus. III. The methylene blue technique and some of its implications. *J. Morph.*, **75**: 261–289.

OBSERVATIONS ON THE RESPIRATION OF AUSTRALORBIS GLABRATUS AND SOME OTHER AQUATIC SNAILS

THEODOR VON BRAND, M. O. NOLAN, AND ELIZABETH ROGERS MANN[1]

Division of Tropical Diseases, National Institute of Health, United States Public Health Service, Bethesda, Maryland

Trematode diseases are best eradicated, or at least reduced in their incidence, by interrupting the life cycle of the parasites in the intermediate hosts, that is, in the case of human infections, by instituting campaigns against the snails harboring the juvenile worms. This has been attempted to date by methods founded purely on empirical findings. It seems probable that a study of the physiology of the snails might yield important clues for the development of chemical means of control.

From a theoretical standpoint it appears likely that snails may be killed by the use of compounds interfering with the cellular respiratory mechanisms. As a preliminary to such work, a study was initiated of the respiration of some fresh water snails. We report in the present paper the results of our experiments on the normal aerobic respiratory physiology of *Australorbis glabratus*, the intermediate host of *Schistosoma mansoni* in the West Indies and South America. Included also are some data on the respiration of other snail species, most of them belonging to genera transmitting trematodes of man or lower animals.

Material and Methods

The following species of snails were employed:

1. Pulmonates

Planorbidae: Australorbis glabratus, laboratory reared from Venezuelan specimens; *Helisoma duryi*, in part specimens freshly collected at Kenilworth Gardens, Md., in part laboratory reared specimens derived from this stock; *Tropicorbis obstructus* and *T. donbilli*, both laboratory reared from specimens collected in Texas.

Lymnaeidae: Lymnaea stagnalis, collected from Mullet Lake, Michigan; *L. palustris* from Stemple Creek, Marin County, California; *L. obrussa* from a canal in the vicinity of Washington, D. C. These snails were used shortly after their arrival in Bethesda.

Physidae: Physa gyrina, in part specimens freshly collected at Kenilworth Gardens, Md., in part laboratory reared specimens derived from this stock; *Physa* sp. specimens freshly collected in a creek near Bethesda, Md., and laboratory reared specimens derived therefrom.

[1] The authors are indebted to Dr. E. G. Berry for the determination of many of the snails and for advice concerning their care, to Miss Ruth Rue and Dr. L. Olivier for the contribution of a number of specimens, and to Mr. Benjamin Mehlman for technical assistance during the respiration experiments.

2. Operculates

Amnicolidae: Amnicola limosa, collected in a canal near Washington, D. C.; *Oncomelania quadrasi,* laboratory reared specimens derived from snails collected in the Philippines; *O. nosophora,* used some weeks after having been received from Japan.

Viviparidae: Campeloma sp., used shortly after having been shipped from Douglas Lake, Michigan.

Melaniidae: Semisulcospira sp., laboratory reared specimens, derived from snails collected in the Philippines.

Pomatiopsidae: Pomatiopsis lapidaria, freshly collected specimens from Fairfax County, Virginia.

Pleuroceridae: Pachychilus sp., specimens collected in Guatemala and kept prior to the determinations for months in an aquarium.

Littorinidae: Littorina irrorata, recently collected from Wicomico River, Maryland.

All species, with the exception of *Littorina,* are fresh water species; *Littorina* occurs in brackish water.

With the exception of the very few cases in which entirely freshly collected specimens were used, the truly aquatic snails were kept in balanced aquaria in a room with a minimum temperature of 21° C. During the summer months the temperature rose to nearly 30° C. The snails were abundantly fed with lettuce leaves and fish food. From time to time some powdered calcium carbonate was added to the water. Those species (*Oncomelania, Pomatiopsis*) that lead in nature a semi-aquatic life were kept in aquaria simulating as best as possible their normal habitat (see Ward, Travis, and Rue, 1947, for details); their chief food consisted of leaf mold.

Before the snails were used for the actual determinations, the water adhering to the specimens was removed with filter paper; they were then weighed on an analytical balance to the nearest mg. The standard temperature used in all experiments, with the exception of those in which the temperature influence was studied, was 30° C. This temperature was slightly higher than that reached by the aquarium water during the summer months and was chosen in order to allow an adequate control of the water bath. This temperature was well tolerated by all species employed.

The respiratory medium was, during the first months of the study, filtered river water. Later on tap water was employed; this was allowed to stand for a minimum of 24 hours in the laboratory to permit the chlorine to evaporate. No difference in respiratory rate was noticed in either type of water.

The respiratory exchange was studied by means of Warburg manometers. The number of snails used for a single determination, the amount of water introduced into the flasks, the size of flasks employed, and the interval between readings varied, depending upon the size of the snails. Up to 16 specimens of the small species, or the juveniles of larger species, were used for each flask. The total capacity of these flasks was about 17 cc., and 2 cc. of water were used as respiratory medium. Of the largest species, single specimens were studied in flasks having a total capacity of about 120 cc.; in these cases 6 cc. of water served as medium. In this latter

case an equilibration period of one hour was necessary before the manometers could be closed while 20 minutes were sufficient when the smaller flasks were employed Depending upon the respiratory rate and the size of the flasks, readings were taken every 15 or 30 minutes for periods of two to four hours. The manometers were shaken with an amplitude of 4 cm. 100 times per minute. Most of the snails remained very quiet in the vessels; only the *Physa* species had a tendency to leave the water and to affix themselves to the sides of the flask.

In most cases the oxygen uptake alone was studied, the carbon dioxide being absorbed in the customary manner by means of 10 per cent KOH. In most series atmospheric air served as gas phase. In these series we were interested in the oxygen consumption of the snails in air saturated water, or, what amounts nearly to the same, that taken by pulmonates from the air itself. The question of the influence of varying oxygen tensions was studied only with *Australorbis glabratus*. In these experiments the gaseous atmosphere was changed by passing gases of known composition for a period of 20 minutes through the manometers. Details will be given in a later section.

The respiratory quotient was studied in some series of *Australorbis glabratus*. Since experience in other experiments had shown that the respiration of these snails stays fairly constant over long periods of time, a modification of Warburg's direct method was employed. The snails were first introduced into flasks containing no KOH and the change in manometer reading was followed for one hour or an hour and a half. The flasks were then removed from the manometers, KOH and filter paper were introduced into the center well and, after reequilibration to the temperature of the water bath, a second set of readings was taken for an equal period as above. The error due to possible changes in the respiratory rates is small since only such experiments were used in which the two sets of readings gave steady values. A few experiments in which either the first or the second set gave inconsistent readings were rejected. The snails used in these series were handled as little as possible; they were not dried or weighed in order to avoid injuries to the shell. This is a very important point since a pronounced carbon dioxide retention sets in very rapidly after the shell has been cracked. The readings taken in these series were calculated as mm^3. oxygen or carbon dioxide per one snail instead of being related to weight or surface area and the RQ was calculated from these values.

Results

1. Relations between size and respiratory rate

The snails varied considerably in size due both to species differences and to differences in age of specimens of one species. This gave an opportunity to study the relation between size and rate of oxygen consumption insofar as both inter- and intraspecific comparisons are concerned. The experiments summarized in this section were carried out from May to August; there was no indication that during this time seasonal variations in oxygen consumption occurred. The determinations were in a few cases carried out with freshly collected specimens; in most cases well fed aquarium snails were employed. This point is important. It will be shown in a later section that the respiratory rate of snails declines very rapidly during starvation. It was even repeatedly noted that determinations carried out on Mondays yielded somewhat lower values than during the remaining days of the week, the

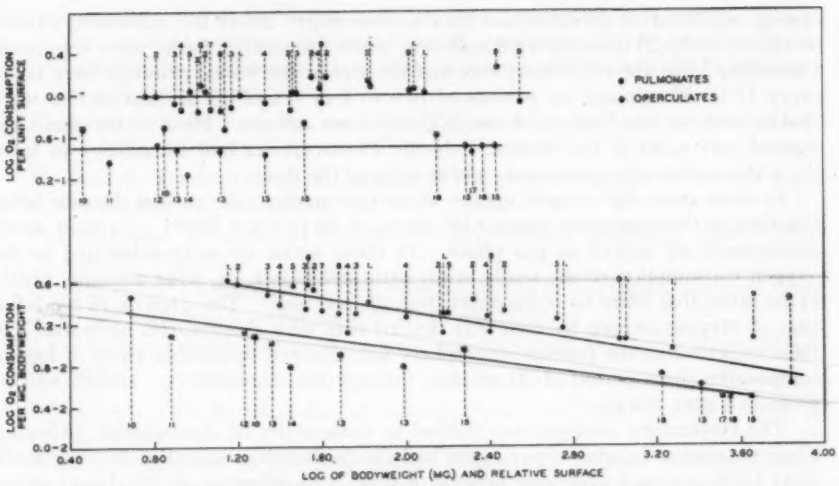

LOG O_2 CONSUMPTION PER UNIT SURFACE
LOG O_2 CONSUMPTION PER MG. BODYWEIGHT
PULMONATES
OPERCULATES
LOG OF BODYWEIGHT (MG.) AND RELATIVE SURFACE

FIGURE 1. Relation between size and rate of oxygen consumption in various species of aquatic snails. Pulmonates: 1. *Australorbis glabratus*, 2. *Physa* sp., 3. *Physa gyrina*, 4. *Lymnaea palustris*, 5. *Helisoma duryi*, 6. *Lymnaea obrussa*, 7. *Tropicorbis donbilli*, 8. *Tropicorbis obstructus*, 9. *Lymnaea stagnalis*. Operculates: 10. *Amnicola limosa*, 11. *Oncomelania quadrasi*, 12. *Pomatiopsis lapidaria*, 13. *Semisulcospira* sp., 14. *Oncomelania nosophora*, 15. *Campeloma* sp., 16. *Pachychilus* sp., 17. *Littorina irrorata*.

TABLE 1

Respiration of Australorbis glabratus of various sizes at 30° C.
Mean values and, in parentheses, extremes

Number of experiments	Number of snails	Weight of single snail mg.	Mm.³ oxygen consumed by 1 snail in 1 hour	Relative surface	Ratios of		
					Weight	Oxygen consumption	Surface
6	40	14 (12, 16)	5.9 (5.4, 6.2)	5.8	1	1	1
6	24	66 (55, 76)	16.8 (14.0, 20.2)	16.3	4.7	2.8	2.8
6	12	153 (122, 201)	30.3 (25.1, 38.4)	28.6	11	5.1	4.9
6	24	564 (525, 601)	81 (60, 95)	68.3	40	14	12
6	6	1137 (1000, 1288)	110 (84, 156)	109	81	19	17
6	6	1903 (1646, 2220)	164 (105, 284)	154	136	28	27

reason being that the snails had more or less exhausted their food supply over the week end.

The data summarized in Figure 1 are average figures from 4 to 12 determinations each, in most cases from 6 determinations. The sizes of the snails are also average values. The data for *Australorbis glabratus* (Table 1) may serve to illustrate these points and the variability introduced by them.

The data, as shown in Figure 1, prove three facts. First, the respiratory rate of pulmonates was without exception higher than that of operculates when snails of equal weight were compared. Second, the respiratory rate of both pulmonates and operculates was inversely correlated to the size of the specimens when calculated on the basis of weight. The slope of the straight lines around which the values of the two groups fluctuated was almost identical, indicating that about the same percentage decline in respiratory rate with increasing weight occurred in both groups. Third, it is quite apparent that straight horizontal lines resulted if the respiratory rates were calculated on the basis of relative surface ($Weight^{2/3}$) rather than weight.

Several reasons may be responsible for the deviations from the average straight lines which in some cases were rather marked. Although we tried to use for the experiments summarized in this section only well fed snails, it is entirely possible that not all species were equally near an optimum diet. Another point is that only the soft parts of the snails are actively metabolizing while our data are based on the complex soft parts plus shell. It was for various reasons not possible to determine in each experiment the shell weight and therefore no attempt was made to correlate the oxygen figures to the weight of the soft parts. Sixteen determinations of the shell weight were carried out at various times during the present experiments on various species; it was found that it varied between 11.2 and 25.4 per cent. It is, of course, also possible that the metabolic rate of various species is inherently somewhat different, even if nutrition and all other factors were exactly equal. Despite these deviations, the trend of the curves is convincing.

It should be mentioned as especially noteworthy that small and large specimens of a given species followed rather closely the surface law, as shown in Table 1 on the example of *Australorbis glabratus*. This is remarkable since in many other organisms juvenile specimens show a higher rate of metabolism than would be expected from this relationship. It is pertinent to mention that the australorbids used for these experiments were all taken from a single aquarium and that the experiments with them were all carried out within two weeks. We were therefore dealing with an exceptionally uniform material insofar as food and physical environmental factors were concerned.

2. Influence of oxygen tension

The experiments on the influence of oxygen tension on the oxygen consumption were carried out with *Australorbis glabratus* specimens of two sizes, small snails weighing 30 to 40 mg. each and medium sized snails weighing 300 to 400 mg. each. Fully grown specimens could not be used because the gaseous atmosphere could not be changed conveniently in the large flasks that alone would accommodate them.

The rate of oxygen consumption of the snails was first established at the oxygen tension of atmospheric air for a period of 1¾ to 2 hours with readings taken at 15 minute intervals. A gas mixture of known oxygen tension was then passed for 20

minutes through the flasks. After the manometers had been closed, the rate of oxygen consumption at the experimental tension was followed for two hours. The temperature was in all experiments 30° C. Twelve experiments were carried out for each of the two groups at each of the four experimental oxygen tensions, and each time new snails were employed.

The summary of all experiments (Table 2) indicates that the reactions of both size groups to changes in oxygen tension were very nearly identical. It is probably justifiable to assume that fully grown specimens would have reacted in an essentially

TABLE 2

Influence of oxygen tension on the oxygen consumption of Australorbis glabratus at 30° C. Mean values and, in parentheses, extremes

Series	O_2 consumption at 160 mm. Hg. tension mm.³/1 gm./1 hr.	Experimental O_2 tension mm. Hg.	O_2 consumption at experimental tension	
			Mm.³/1 gm./1 hr.	Per cent of 160 mm. value
A	296 (254, 318)	760	288 (250, 334)	98±8.0
B	140 (101, 177)	760	144 (97, 190)	104±3.1
A	255 (226, 270)	38	205 (186, 221)	81±2.0
B	161 (122, 187)	38	138 (99, 178)	86±2.3
A	311 (249, 416)	13	260 (234, 279)	85±3.2
B	163 (129, 183)	13	156 (110, 204)	95±4.1
A	246 (187, 310)	5	29.6 (8.2, 49.2)	12±0.4
B	177 (147, 262)	5	12.5 (3.8, 17.3)	7±0.7

Series A: Snails weighing 30 to 40 mg. each.
Series B: Snails weighing 300 to 400 mg. each.

identical manner. On the whole, there was little indication that the oxygen consumption was markedly influenced by changes in tension between 760 and 13 mm. Somewhere below this latter tension the consumption began to fall off rapidly; at a tension of 5 mm. only a small fraction of the normal amount was consumed. The data obtained prove conclusively that *Australorbis glabratus* belongs to the group of invertebrates capable of maintaining a more or less uniform rate of oxygen consumption over a wide range of tensions.

3. Influence of temperature

The influence of temperature on the rate of oxygen consumption of *Australorbis glabratus* was investigated in the range of 0.3 to 41° C. The experiments were carried out with fairly small snails, the individual specimen weighing from 40 to 90 mg. Six snails were introduced into each flask and 12 experiments were conducted for each experimental temperature. New snails were used for each experiment. The respiratory rate of every lot was first established at our standard temperature of 30° C., four readings being taken at 15 minute intervals. The manometers were then transferred to a second water bath regulated to the intended experimental temperature. After equilibration the respiration was followed for a two hour period with readings taken at 15 or 30 minute intervals at the higher and lower temperatures respectively. The manometers were then transferred back to the original 30° C. water bath and a new set of readings was taken at 15 minute intervals in order to test whether during this recovery period the original level of oxygen consumption would again be reached.

The absolute values obtained in the various series during the initial 30° C. period varied somewhat, due probably to the differences in size between snails of the

TABLE 3

Influence of temperature on the oxygen consumption of Australorbis glabratus, averages and, in parentheses, extremes

Initial oxygen consumption at 30° C. mm.³ O_2/1 gm./1 hr.	Experimental temperature °C.	Oxygen consumption at experimental temperature		Oxygen consumption at 30° C. following experimental temperature			
				First hour		Second hour	
		Mm.³ O_2/1 gm./1 hr.	Per cent of initial value	Mm.³ O_2/1 gm./1 hr.	Per cent of initial value	Mm.³ O_2/1 gm./1 hr.	Per cent of initial value
151 (119, 186)	0.3	6.6 (3.7, 10.0)	4.3±0.25	58 (39, 74)	39±2.9	111 (73, 151)	75±6.9
222 (169, 260)	5.0	15.2 (11.8, 17.4)	6.9±0.34	131 (101, 162)	60±4.0	225 (185, 290)	102±4.7
208 (155, 256)	10.0	21.3 (16.7, 27.9)	10.2±0.16	204 (166, 253)	98±1.9		
184 (136, 224)	14.8	48.3 (40.0, 54.3)	27±1.3	178 (167, 196)	99±5.0	175 (150, 222)	97±4.9
179 (141, 234)	19.7	88 (76, 96)	50±2.2	185 (158, 214)	105±3.9		
175 (134, 200)	24.7	115 (94, 140)	67±3.5	168 (145, 188)	97±2.9		
193 (139, 228)	37.0	281 (226, 358)	148±7.0	193 (138, 260)	99±3.8		
225 (190, 282)	41.0	158 (111, 221)	71±3.7	152 (86, 248)	68±7.2		

various batches and to small differences in their nutritional state. These differences do not, however, interfere with an evaluation of the experiments since the figures found during exposure to the experimental temperatures and those obtained during the recovery period could be expressed in per cent of the initial value, thus eliminating any influence of these variations.

The oxygen consumption of the snails (Table 3) increased in the range 0.3° to 37° C. At 41° C., however, the animals were definitely damaged. Their respiratory rate decreased, and it did not come back to the original level during the recovery period. After the end of the recovery period, the snails were kept in beakers over night at room temperature and it was found that all were dead the following morning. The lowest temperature employed, 0.3° C., was also damaging. The respiratory rate increased only slowly after the snails had been transferred back to 30° C., and, after being kept over night at room temperature, about half the snails were dead. All other temperatures were well tolerated and the respiration returned during the recovery period to the pre-experimental value.

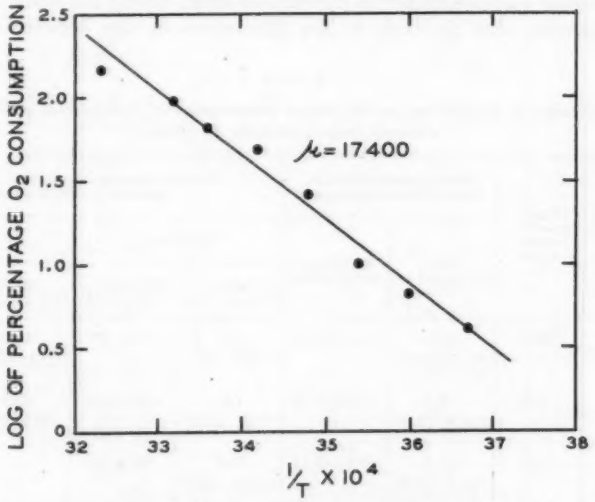


FIGURE 2. Temperature relationships of the oxygen consumption of *Australorbis glabratus* in the range 0.3° to 37° C. expressed according to Arrhenius' equation.

Using the percentage oxygen figures, the temperature relationship was then calculated according to Arrhenius' equation (Fig. 2) for the range 0.3° to 37° C. A single straight line was obtained and the μ value of 17,400 is entirely within the normal range.

Upon projection of the percentage figures on Krogh's (1914) normal curve a very satisfactory agreement to this curve was obtained (Fig. 3). Krogh's curve has been established only for the range 0° to 29° C. The points obtained in the present investigation beyond this range show an excellent fit to an extension of this curve. It would seem possible that this extension may have the same general applicability as the original curve.

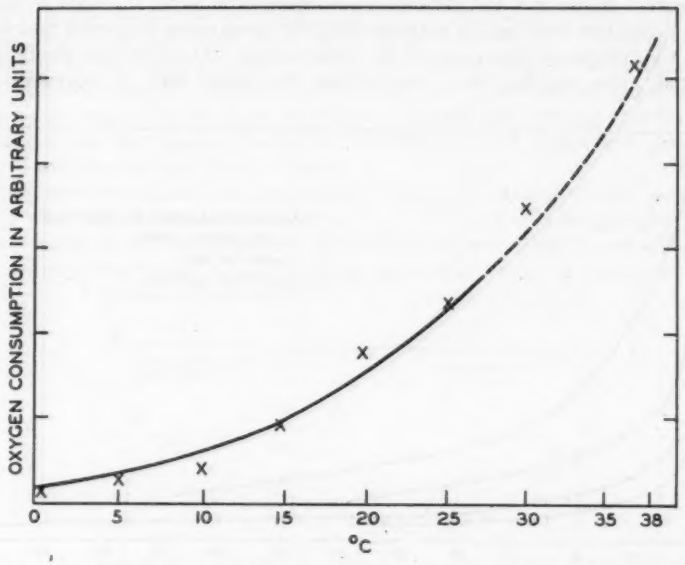


FIGURE 3. Projection of the percentage oxygen figures of *Australorbis glabratus*, taken from Table 3, on Krogh's normal curve and extension of this curve to 37° C.

4. Influence of starvation

The influence of starvation on the rate of oxygen consumption was studied with *Australorbis glabratus, Helisoma duryi, Physa* sp., and *Physa gyrina.* Of the three former species six groups of two snails each were employed while six groups of four snails each were used of *P. gyrina.* The snails of each species were selected as to uniformity in size. Each group was kept in a beaker with about 200 cc. water which was changed at frequent intervals and which was aerated by a slow stream of air bubbling through the water. The experiments were carried out during the summer months, the beakers being kept in a room without temperature control. The water temperature was, however, checked daily. It varied between 21.0° and 29.5° C.; the average temperature was about 27° C. The rate of oxygen consumption was determined for each group at the start of the starvation period, and after designated periods of starvation; the temperature during the actual determinations was 30° C.

The snails were weighed before each determination. The average loss in weight towards the end of the experiments was 18 per cent of the original weight in the case of *Physa* sp., 13 per cent both in *Australorbis glabratus* and *Physa gyrina,* and only 3 per cent in *Helisoma duryi.* Why this latter species lost relatively so little weight is not clear; it was the species that resisted starvation longest. Because this loss in weight would mask to a certain extent the decrease in metabolic level, if the oxygen values would have been calculated on the basis of the weight the organisms had on a specified day, the values are expressed in mm^3. oxygen/1 snail/1 hour.

Figure 4 shows that the various series ended after three to seven weeks starvation. When one snail out of a group died, the group was discarded and the whole series was completed when one of the snails of the last remaining group had died. Obviously then, our figures do not indicate the upper limit of starvation that the

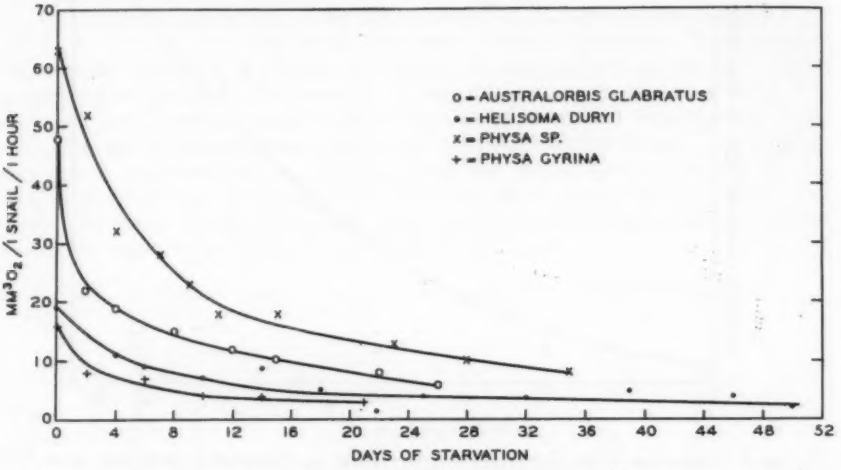


FIGURE 4. Influence of starvation on the rate of oxygen consumption of four species of pulmonate snails, absolute figures.

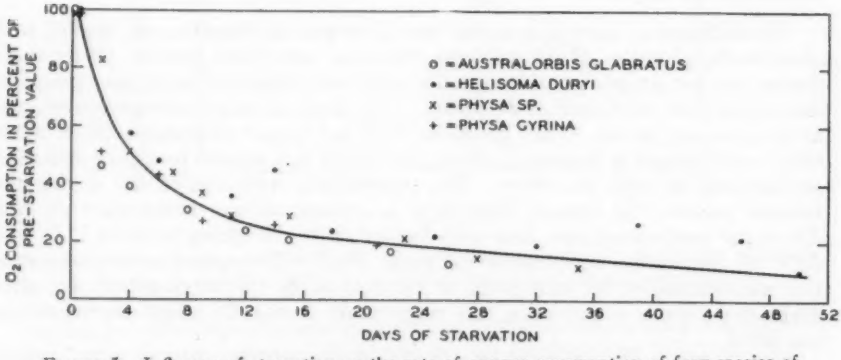

o = AUSTRALORBIS GLABRATUS
• = HELISOMA DURYI
x = PHYSA SP.
+ = PHYSA GYRINA
O₂ CONSUMPTION IN PERCENT OF PRE-STARVATION VALUE
DAYS OF STARVATION

FIGURE 5. Influence of starvation on the rate of oxygen consumption of four species of pulmonate snails, percentage figures.

various species can endure at the temperature prevailing in our experiments. We are even hesitant to assume that all our snails died of actual starvation; the unavoidable repeated handling may well have hastened the end of one or the other specimen.

It is obvious (Fig. 4) that in all four species the rate of oxygen consumption declined sharply during the initial stages of starvation. Later the decline was much

less marked, but no completely steady level was reached. In order to test whether the influence of starvation was noticeably different in the various species, the starvation values were then calculated in per cent of the initial values. This eliminated the differences in the absolute amounts due to the various sizes of the snails belonging to the different species. Figure 5 shows that the values thus obtained fit fairly well to a single curve; really large deviations occurred only during the first days of starvation. On the whole it is evident that the effect of progressive starvation was quite similar in the four species studied.

The respiratory quotient was studied only in the case of *Australorbis glabratus*. The snails were kept in these experiments in a room with an average temperature of approximately 25° C. but the actual RQ determinations were carried out at our standard temperature of 30° C. The points shown on Figure 6 are mean values of from five to 14 determinations each.

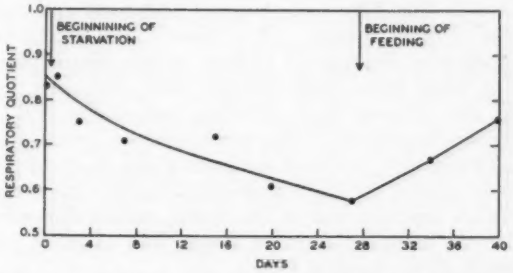


FIGURE 6. Respiratory quotient of *Australorbis glabratus* under the influence of starvation and subsequent feeding.

The respiratory quotient of well fed snails was around 0.85; the values found during the progressive stages of starvation fluctuated around the curve shown in Figure 6. A progressive lowering is evident and after three to four weeks starvation the surprisingly low value of 0.6 was reached. After 27 days inanition the snails were again fed and the respiratory quotient determined after one and two weeks feeding. It did rise during this period markedly but failed to reach the pre-starvation level.

DISCUSSION

The present investigation has shown that aquatic pulmonate snails have consistently a higher rate of oxygen consumption than operculates of the same size. A definite reason for this difference could not be adduced; it is an illustration of the well known fact that animals with different organization frequently have different metabolic levels.

In both groups the respiratory rate was inversely correlated to the weight of the specimens, but remained more or less constant if referred to relative surface. This relationship held true both in intraspecific and interspecific comparisons and was especially close in the former case. The question of the relationships between body size and metabolic rate has recently been reviewed by von Buddenbrock (1939), Kleiber (1947), and Zeuthen (1947). While the surface law applies rather

closely in the case of most vertebrates, the matter is more complex in invertebrates, or when the animal kingdom as a whole is considered. Large deviations are especially apparent in the largest and smallest organisms for which data are available. Insofar as molluscs specifically are concerned, Weinland (1918) found a positive correlation between surface and respiratory rate in *Anodonta*, while Liebsch (1928) denies such a relationship in terrestrial snails; he found a positive correlation between weight and respiratory rate. It should be pointed out, however, that the nutritional state of his snails was not uniform and that the size range of his specimens was appreciably smaller than that employed by us. An inspection of our data (Fig. 1) shows that within limited size ranges lines apparently showing a constancy of weight/O_2 relationship could be drawn but that the over all picture is distinctly against the validity of such a procedure. In view of the present results, it would be interesting to reinvestigate the terrestrial snails on a broader basis; it would be rather remarkable if they differed so fundamentally from the aquatic species.

It is true, however, that differences between terrestrial and aquatic snails have been reported also in other respects. It is thus a well established fact that the former show a pronounced dependency of their oxygen consumption on the oxygen tension (Thunberg, 1905; Dahr, 1927; Fischer, 1931; Harnisch, 1932) while many marine snails do not (Moore, Edie, Whitley, and Dakin, 1912; Raffy, 1933). *Australorbis glabratus* belongs, according to the present investigation, to this latter group. This, unquestionably, is due to the haemoglobin in its blood. Although the oxygen dissociation curve of the *Australorbis* haemoglobin has not yet been studied, it can be presumed to resemble that of the closely related *Planorbis*. The oxygen affinities of the latter's haemoglobin make it especially suited to procure oxygen at low tensions (Leitch, 1916; Borden, 1931).

The fact that *Australorbis* can hold its oxygen consumption at a normal level even at relatively low tensions may have a bearing on control measures. The application of some chemicals to snail infested waters results in the snails' burrowing into the mud and so escaping the direct action of the poison (W. H. Wright, personal communication). Although it is not known whether *Australorbis glabratus* specifically reacts in this way, it must be expected that snails with similar respiratory characteristics would not be easily killed by asphyxiation simply by being driven into oxygen-poor surroundings. Under very low oxygen tensions, it is true, the oxygen consumption is markedly lowered. It must be remembered, however, that snails in general are endowed with fairly well developed anaerobic functions (summary of the literature in von Brand, 1946).

The temperature relationships of *Australorbis glabratus* correspond to those commonly found in other invertebrates. The range of temperatures tolerated at least for the short periods employed in the experiments was rather wide. The variations in metabolic level encountered in this range may well have to be taken into consideration in control measures directed against this schistosomiasis-carrying species. Due to the lower metabolic level the snails will consume less food at lower temperatures than at higher ones. A poison, therefore, that acts via the alimentary canal may have to be kept longer at a given concentration in the cooler headwaters of a stream than in the warmer lower regions in order to insure that it reaches adequate concentrations within the tissues of the snail. It should be noted in this connection that according to Luttermoser (1947) in Venezuela at least "the only

workable method for eradicating the snails was to eliminate them in the headwaters and to destroy them progressively downstream." Very likely, however, the temperature range in schistosomiasis-endangered river systems will not be quite so broad as the extremes employed in our laboratory experiments.

The influence of starvation on the oxygen consumption of *Australorbis glabratus, Helisoma duryi, Physa gyrina,* and *Physa* sp. was pronounced and quite similar in the four species. A progressive lowering in metabolic level occurred until the snails finally died. No steady rate of oxygen consumption was reached, the snails resembling in this respect starving warm-blooded animals (Krogh, 1916).

The respiratory quotient of *Australorbis glabratus* sank during starvation from an initial value of 0.85 to the surprisingly low level of around 0.6. Even lower values have been observed by Bellion (1909) in *Helix* and by Liebsch (1928) in several species of terrestrial pulmonates. *Helix* was studied towards the end of hibernation, that is, after having starved for a long time; Liebsch's specimens were probably at least semi-starving. The interpretation of the respiratory quotients of animals having calcareous shells is notoriously difficult; the following interpretation can therefore be only tentative. In view of the fact that the occurrence of glycogen has been demonstrated in *Australorbis glabratus* (von Brand and Files, 1947), it does appear probable that the relatively high RQ of well fed snails is due to the utilization of this polysaccharide. The glycogen reserve does not seem to last for a long time; soon values typical for fat and protein consumption are reached. The very low values found in the last stages may indicate either the new formation of carbohydrate from protein or, possibly, fat, or they may be due to carbon dioxide retention. The data at hand do not permit a decision between these possibilities.

The respiratory quotient of australorbids fed again after a starvation period of four weeks rose but failed to reach in two weeks the original level. Before starvation, the snails had been kept in a balanced aquarium; during and after the inanition period they were kept isolated in pairs in beakers. It is possible that they had in the aquarium some accessory food material at their disposal that was lacking in the beakers. But it is equally possible that during the recovery period a certain amount of carbon dioxide retention took place in connection with restitution processes on the shell. We gained at least in some cases the impression that the shells became brittle during protracted periods of starvation but we do not have quantitative data proving this point and emphasize that we do not consider it as more than a possibility. It may be mentioned that during hibernation, which of course corresponds to a starvation period, movements of inorganic substances between soft parts and shell likely occur in the case of *Helix* (von Brand, 1931).

Summary

1. A study of the rate of oxygen consumption of nine species of pulmonate snails and eight species of operculate snails showed that the pulmonates had consistently a higher metabolic level than the operculates if specimens of equal weight were compared.

2. In both groups, the intensity of oxygen consumption decreased with increasing size of the specimens if referred to unit weight, but remained about constant if referred to relative surface. The oxygen/surface relationship held true both in inter- and intra-specific comparisons and was especially close in the latter case.

3. *Australorbis glabratus* was able to maintain an approximately steady rate of oxygen consumption over a wide range of oxygen tensions.

4. The oxygen consumption of *Australorbis glabratus* increased with rising temperature in the range of 0.3 to 37° C., but 41° C. was lethal. The temperature relationship calculated according to Arrhenius' equation gave within the tolerated temperature range a straight line. A good fit to Krogh's normal curve was also obtained and an extension of this curve to a higher temperature range than used by Krogh is presented.

5. The intensity of the oxygen consumption of four species of pulmonate snails sank during protracted starvation first rapidly and later on slowly without reaching a steady level. The respiratory quotient of *Australorbis glabratus* sank during inanition to very low levels and rose only slowly after feeding was begun again.

6. The possible implications of some of the studied factors on snail control measures are briefly discussed.

LITERATURE CITED

BELLION, M., 1909. Contribution à l'étude de l'hibernation chez les invertébrés. Recherches expérimentales sur l'hibernation de l'escargot (*Helix pomatia* L.). *Ann. Univ. Lyon*, N. S. *1*, Fasc. **27**: 1–139.

BORDEN, M. A., 1931. A study of the respiration and of the function of haemoglobin in *Planorbis corneus* and *Arenicola marina*. *J. Marine Assoc. Un. Kingdom*, N. S. **17**: 709–738.

DAHR, E., 1927. Studien ueber die Respiration der Landpulmonaten. *Lunds Univ. Aarsskrift*, N. F. Avd. 2, **23**: 1–120.

FISCHER, P. H., 1931. Recherches sur la vie ralentie de l'escargot (*Helix pomatia* L.). *J. Conchyliologie*, **75**: 1–200.

HARNISCH, O., 1932. Studien zur Physiologie des Gaswechsels von Tieren ohne Regulierung der Sauerstoffaufnahme bei wechselndem O_2-Partialdruck. I. Die Sauerstoffaufnahme. *Ztschr. Vergl. Physiol.*, **16**: 335–344.

KLEIBER, M., 1947. Body size and metabolic rate. *Physiol. Rev.*, **27**: 511–541.

KROGH, A., 1914. The quantitative relation between temperature and standard metabolism in animals. *Ztschr. Physik.-chem. Biol.*, **1**: 491–508.

KROGH, A., 1916. *The respiratory exchange of animals and man*. London.

LEITCH, I., 1916. The function of haemoglobin in invertebrates with special reference to *Planorbis* and *Chironomus* larvae. *J. Physiol.*, **50**: 370–379.

LIEBSCH, W., 1928. Ueber die Atmung einiger Heliciden Eine Untersuchung zum Oberflaechengesetz. *Zool. Jahrb. Abt. Allg. Zool. und Physiol.*, **46**: 161–208.

LUTTERMOSER, G. W., 1947. The control of the blood-fluke disease (schistosomiasis) in Venezuela. Inst. Inter-Amer. Affairs. Health and Sanitation Div. Newsletter, Oct. 1947.

MOORE, B., EDIE, E. S., WHITLEY, E., AND DAKIN, W. J., 1912. The nutrition and metabolism of marine animals in relationship to (a) dissolved organic matter and (b) particulate organic matter of sea-water. *Biochem. J.*, **6**: 255–296.

RAFFY, A., 1933. Recherches sur le métabolisme respiratoire des poikilothermes aquatiques. *Ann. Inst. Océanogr. Paris*, Ser. IV, **13**: 263–393.

THUNBERG, T., 1905. Der Gasaustausch einiger niederer Thiere in seiner Abhaengigkeit vom Sauerstoffpartiardruck. *Skand. Arch. Physiol.*, **17**: 133–195.

VON BRAND, T., 1931. Der Jahreszyklus im Stoffbestand der Weinbergschnecke *Helix pomatia*. *Ztschr. Vergl. Physiol.*, **14**: 200–264.

VON BRAND, T., 1946. Anaerobiosis in invertebrates. *Biodynamica Monogr.* No. 4. Normandy, Missouri.

VON BRAND, T., AND FILES, V. S., 1947. Chemical and histological observations on the influence of *Schistosoma mansoni* infection on *Australorbis glabratus*. *J. Parasitol.* **33**: 476–482.

VON BUDDENBROCK, W., 1939. Grundriss der vergleichenden Physiologie. 2nd. ed. Vol. *2*. Berlin.

WARD, P. A., TRAVIS, D., AND RUE, R. E., 1947. Methods of establishing and maintaining snails in the laboratory. *National Inst. Health Bull.* No. **189**: 70–80.

WEINLAND, E., 1918. Beobachtungen ueber den Gaswechsel von *Anodonta cygnea* L. Nach gemeinsam mit Frl. Vernette L. Gibbons und Herrn Adolf Adams angestellten Versuchen. *Ztschr. Biol.*, **69**: 1–86.

ZEUTHEN, E., 1947. Body size and metabolic rate in the animal kingdom with special regard to the marine micro-fauna. *C. R. Trav. Lab. Carlsberg Ser. Chim.*, **26**: 17–161.

THE FUNGUS LAGENIDIUM CALLINECTES COUCH (1942) ON EGGS OF THE BLUE CRAB IN CHESAPEAKE BAY[1]

R. ROGERS-TALBERT[2]

Virginia Fisheries Laboratory, Yorktown

In 1941 a parasitic fungus was first observed on the eggs of many blue crabs obtained from natural spawning areas in Chesapeake Bay (Sandoz, Rogers, and Newcombe, 1944). Dr. John N. Couch of the University of North Carolina kindly examined infected samples and recognized the parasite to be a new species, which he named *Lagenidium callinectes* Couch (1942).

Marked annual fluctuations in the commercial catches of the blue crab *Callinectes sapidus* Rathbun (1895) have taken place. The discovery of the egg parasite raised the questions of how it affects the development of the crab embryo, what percentage of the eggs of a crab may be infected, and how widely the parasite is distributed in Chesapeake Bay. Conceivably, such a parasite could constitute a serious biological factor limiting the production of crab larvae and causing, at least in part, yearly fluctuations in the commercial population.

Laboratory and field studies were carried out: (1) to ascertain the conditions of existence of the parasite in the individual crab eggs as well as on and in the egg mass (commonly called "sponge"); (2) to show how readily infection may be transmitted under certain conditions; (3) to indicate the effects of salinity and temperature on the survival and development of the fungus; and (4) to show the areas of Chesapeake Bay in which it occurs and the approximate degree of infection.

Acknowledgment is made to all persons who aided in this study. Special thanks are expressed to Professor John N. Couch of the University of North Carolina, who identified and described the fungus parasite; to Mr. John C. Pearson, Dr. Sewell H. Hopkins and Mr. R. Winston Menzel for certain crab collections; to Geo. W. Amory, Jr., W. J. Bradshaw, Jr., Chesapeake Crab Co., Costin Co. Inc., G. T. Elliott, Inc., V. S. Lankford, M. F. Quinn, and O. R. Mills for cooperation in making the collections possible; to Mrs. Ruth E. Allen for the use of illustrations; Mrs. Mildred D. Sandoz for helpful counsel; and especially to Dr. Sewell H. Hopkins for valuable criticism of the manuscript. This work was done under the direction of Dr. Curtis L. Newcombe, formerly director of the Virginia Fisheries Laboratory.

Biology of the Blue Crab

The blue crab, *Callinectes sapidus* Rathbun, occurs abundantly in Chesapeake Bay and provides the source for a major seafood industry in the Tidewater section

[1] Joint contribution from the Virginia Fisheries Laboratory of the College of William and Mary and Commission of Fisheries of Virginia (Number 28), and from the Department of Biology of the College of William and Mary.

[2] Present address: Department of Zoology, University of Wisconsin, Madison, Wisconsin. This work was done in partial fulfillment of requirements for the degree of Master of Arts at the College of William and Mary.

of Maryland and Virginia. The annual production of the raw product of these crabs is valued at more than a million dollars.[3]

Churchill (1919) made studies on the life history of this species in Chesapeake Bay. He found that the average blue crab lives two to three years during which time definite migrations take place throughout the bay. In the spawning season, which lasts from May to September of each year, large numbers of egg-bearing females are found in the waters at the mouth of the bay—in the vicinity of Cape Charles and in Hampton Roads (Fig. 1). The gravid female carries her eggs on four pairs of small abdominal appendages (pleopods). These appendages are provided with many hair-like filaments to which the eggs become attached by a glandular secretion when they are extruded from the oviduct (Fig. 2). Incubation is completed in about two weeks in Chesapeake Bay. An egg mass or sponge is estimated to contain about 2,000,000 eggs. The writer has observed that sponges vary a great deal in size, averaging 75 mm. wide, 50 mm. long, and 40 mm. deep. The bulk of so many eggs forces the folded abdomen (apron) away from the ventral side of the cephalothorax until it extends almost posteriorly. Observations have indicated that there is uniform development within a blue crab sponge (Lockhead and Newcombe, 1942), only 1 to 4 per cent of the eggs showing a retarded or undeveloped condition.

The approximate age of crab embryos can be determined by the color of the sponge. A new sponge is bright orange or yellow due to the large amount of yolk material in the egg. With development, the color of the sponge darkens to brown and finally black as the nutrient yolk is used up and pigment spots appear. Thus, age may be designated by three colors: Yellow, representing the first to the fifth day after eggs are deposited; brown, the sixth to the eleventh day; black, the twelfth to the fifteenth day.

Hatching releases zoeal larvae which are abundant in the plankton of the lower bay waters. After passing through at least five zoeal instars (Hopkins, 1944), a second larval stage, the megalops, is attained. There is a single megalops instar [4] which molts directly into the first crab stage. The young crabs begin to migrate up-bay or into the near-by rivers. Such crabs hibernate in these waters during the winter, then complete their development and mate the following summer. After mating, if not before, the females begin a migration to the natural spawning area in the vicinity of the Capes. Many arrive in the lower bay at the end of their second summer. They winter here and produce their eggs when conditions become favorable the following year. A large proportion of the females which mate late in their second summer may winter en route to the capes. Many of them produce their sponges the next summer before they reach the spawning grounds. This partially accounts for the large number of females with yellow sponges and the very few with dark sponges contained in commercial catches of the Egg Island-York Spit area (Fig. 1).

Adult male crabs do not make an extensive southward migration as do the

[3] For the period 1939–1943 the average annual production of raw product in Chesapeake Bay was 42,807,050 lbs., averaging an annual value of $1,327,882. *Fishery Statistics of the U. S.*, Fish and Wildlife Service, U. S. Dept. of Interior.

[4] M. D. Sandoz, in unpublished data on development of the blue crab, Virginia Fisheries Laboratory.

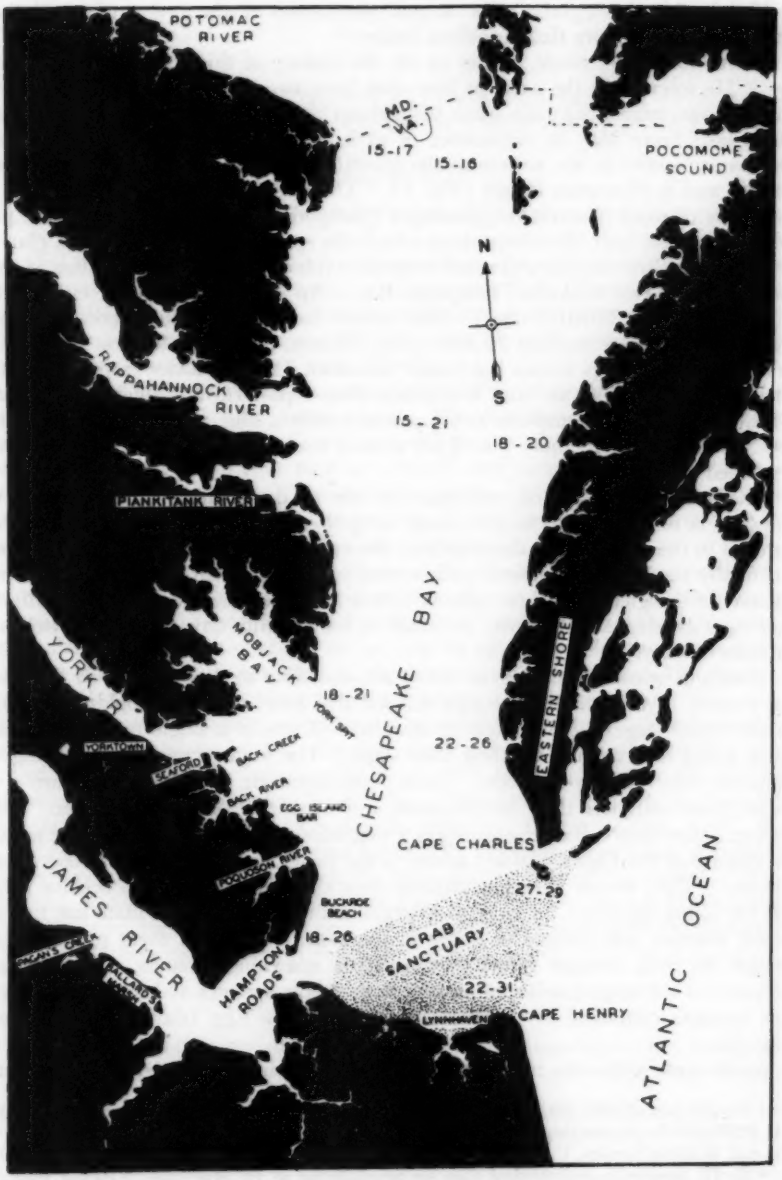


FIGURE 1. Lower Chesapeake Bay, showing the blue crab sanctuary and areas where blue crabs were collected. Average surface-bottom salinity records are given. (After Wells, Bailey, and Henderson, 1929.) Drawn by G. M. Moore.

females but remain in the rivers and bay waters along the entire length of the bay where they have matured.

The migratory habit of the blue crab endows Maryland seafood dealers with a greater proportion of the soft crab industry because the waters of Maryland and adjoining sections of Virginia are more heavily populated with immature crabs which undergo periodic moltings during their growth. The large population of mature hard crabs in the lower bay is responsible for the crab meat canning industry being located primarily at Hampton, Virginia.

FIGURE 2. A segment from the abdomen of a female blue crab. Eggs are borne attached to the longer filaments of the endopodite. (Drawn by R. E. Allen.)

To protect the brood stock of blue crabs, the Commission of Fisheries of Virginia maintains a crab sanctuary (Fig. 1) at the mouth of Chesapeake Bay. Optimum conditions exist here for the development of blue crab eggs and crab fishing is prohibited in these waters during the spawning season. Examinations of egg-bearing crabs from the sanctuary in 1942 indicated that the fungus parasite, *Lagenidium callinectes* Couch, occurred there. This discovery aroused a wide interest among fishermen and conservationists and raised a question as to the value of protecting sponge crabs in the area. Furthermore, it pointed to a need for locating the waters where infection exists in order to determine whether the fungus is a general or localized parasite.

CHARACTERISTICS OF THE FUNGUS

The description of the life history of *Lagenidium callinectes* Couch (1942) has been a valuable aid in this study. In his observations of the organism Couch found that when germination of the zoospore begins, a delicate germ tube is sent through the egg membranes. This tube grows rapidly into a network of branched mycelium that soon fills the entire egg (Fig. 3). From the mycelium, stumpy, thumb-like projections, or hyphae, pass through the egg membranes to the outside (Fig. 4). These hyphae quickly mature into sporangia which rupture and discharge new spores to continue the cycle of infection. When the nutrient material of the egg has been

exhausted by the fungus, the mycelium appears to break up into heavy walled, resting cells that seem to be resistant to adverse conditions. However, neither germination of these cells nor a sexual phase of reproduction has yet been observed. Infected eggs soon give definite indication of being abnormal; they are opaque and dwarfed, the diameter becoming reduced from about 290 micra to approximately 231 micra (Fig. 4) (Couch, 1942).

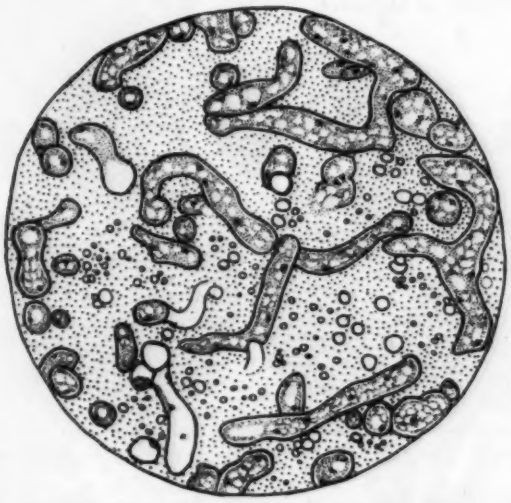


FIGURE 3. Cross section of a blue crab egg parasitized by *Lagenidium callinectes*, showing extensive internal mycelium (400 ×).

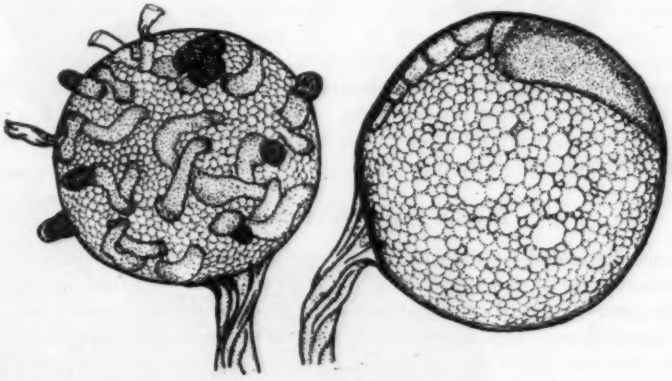


FIGURE 4. Two blue crab eggs from a single pleopod filament (200 ×). The parasitized egg (left) demonstrates 8 external hyphae and 3 empty exit tubes. Internal mycelium is seen through the transparent egg membranes. Parasitized egg shows reduction in size.

The several developmental stages as described by Couch were observed, and it has been possible to maintain the organism under laboratory conditions, thus providing a better understanding of how the parasite destroys the host egg. Among infected eggs collected from Chesapeake Bay and eggs infected under laboratory conditions, the number of external hyphae varies greatly; usually there are one or two on an egg, but frequently nine or more projections are observed at once.

Methods

Studies of this parasitic fungus were carried out by random sampling of sponge crabs and by microscopic examination of the eggs. Lactophenol was used to clear the eggs and expose the mycelium. In this work the age of the sponge was designated by color: yellow, brown, or black.

Preliminary sampling up to and including 1943 indicated the waters of heaviest infection. Early in 1944 weekly sampling of various crabbing areas was begun. The samples, consisting of 20 to 25 sponges each, were preserved in 10 per cent formalin as soon as the commercial boats docked, which was only a few hours at most after the crabs were removed from the water. Collections were made in the Hampton Roads-Lynnhaven and Egg Island-York Spit areas. Relatively few sponge crabs are found north of York Spit.

To determine the extent of sponge infection, several methods were attempted before a satisfactory one was found. At first, eggs were taken at random from the outside of the mass and examined microscopically. A count totaling 500 eggs was made to estimate the percentage of exterior infection. Then about half the sponge was cut away and the procedure repeated, using eggs from the interior. It was found that infection did not penetrate to the interior, so observations were continued only on the peripheral portion of the sponge. Where infection was observed, several filaments were detached at the base and examined for the progression of fungus along the strand. These methods of computing degrees of infection involved a high probability of error in view of the enormous number of eggs per sponge. It was necessary, therefore, to abandon this plan of estimating the percentage of diseased eggs since it was impossible to count enough in every sponge to determine an accurate percentage.

Satisfactory results were obtained by setting up a standard based on visible areas of infected eggs. When the fungus has spread through many eggs in a given area of a sponge, the diseased portion in contrast to normal eggs assumes a brown color on yellow sponges and a grayish color on brown and black sponges. This is due to opacity of the eggs caused by the parasite. The following classification was adopted for differentiating the infected sponges in routine collections:

Slight—fungus present in microscopic examinations but no areas of infection visible to the naked eye.

Moderate—presence of visible areas of infection (which may be one or more) but less than half the sponge visibly infected.

Heavy—more than half of sponge periphery visibly infected, but with one or two small areas where infection has not become heavy enough to be seen.

Very heavy—a complete peripheral infection with no areas of healthy eggs visible.

From all the samples collected, four sponges were selected which demonstrated the different degrees of infection. From each pleopod of these sponges, 25 filaments were detached at the base and examined microscopically. Observations were made on the depth of fungus penetration, the general condition of interior eggs, and the posibilities of an appreciable hatch of larvae despite the exterior coat of infection.

Information on transmission of the fungus was obtained using the following procedures:

1. Several infected and uninfected egg-bearing crabs, selected from commercial catches at Seaford and Hampton, were placed together in aquaria.

2. Healthy and infected eggs from two different sponges were placed at opposite ends of porcelain pans and on opposite sides of large finger bowls. Running water from aquaria containing infected crabs was collected in pans into which normal eggs were then introduced. For controls, healthy eggs were placed in pans of water and females with sponges were placed in aquaria.

3. Infected and uninfected sponges in various stages of development were suspended in the York River near shore (Sandoz, Rogers, and Newcombe, 1944). A small cage ($30 \times 13 \times 25$ cm.) constructed of window screening on a wooden frame was used to protect the sponges and keep them afloat. Individual pleopods, detached from the sponge, were threaded with string near the base of the protopodite and fastened to hooks inside the cage.

To indicate how the salinity factor affects the fungus, a series of salinities ranging from pond water up to the approximate concentration of sea water was prepared, using pond water and salt extracted from York River water. In filaments selected for these salinity tests, the fungus had attacked all eggs within 2 or 3 mm. of the distal end; below this point eggs were developing normally. One filament was placed in each Petri plate of 50 cc. of water.

No other species of crabs has been observed with this infection, so studies were carried out to determine whether or not this parasite has a specific affinity for eggs of *Callinectes sapidus*. Strands of infected blue crab eggs were placed with the healthy eggs of several other species in Syracuse watch glasses containing York River water. The other forms of crabs included the oyster crab (*Pinnotheres ostreum* Say, 1817), the wood crab (*Sesarma cinereum* Bosc, 1801), the mud crab (*Neopanope texiana* Rathbun, 1900), and the spider crab (*Libinia emarginata* Leach, 1815), all of which are found in the area where infected blue crabs occur.

Results and Discussion

Lagenidium callinectes Couch, a peripheral parasite. Microscopic examinations have shown that infection by *L. callinectes* is restricted principally to the periphery of a sponge (Fig. 5). In fungus infected crabs, all eggs from the distal end of the strand down about 3 mm. may become infected, but below this eggs are found to be normal.

Eggs lying in the interior of a sponge are packed closely together. The filaments, which are found only on the posterior side of the pleopods, vary in length from approximately 3 to 22 mm. (Fig. 2). The longer ones extend from the base of the pleopod while the short ones are at the tip. After eggs have been extruded, this variation permits none of the filaments to be buried within the mass. The volume of eggs is so great that the abdomen is pushed away from the cephalothorax

and water flows freely around the outside of the sponge. The outer eggs of the mass serve as buffers for those on the interior, since commensals and parasites come in contact with these outer eggs first. Also, interior eggs lie closer together and do not seem to permit a rapid flow of water within the sponge, the interspaces only being large enough for water to seep slowly around the eggs. This movement of water is further aided by activities of the mother crab, such as vigorous jerking of her abdomen and frequent stirring of the eggs with her walking legs. *L. callinectes* gains a foothold rather quickly, but never seems able to penetrate to great depths

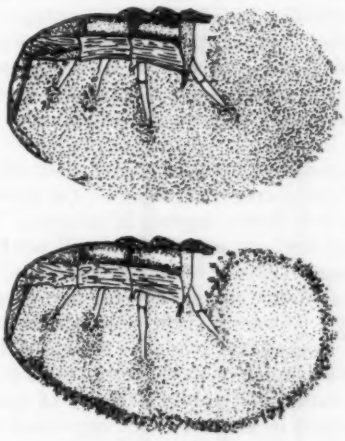


FIGURE 5. Egg masses of *Callinectes sapidus* Rathbun in longitudinal sections, showing a normal (above) and a diseased (below) condition with peripheral infection.

within the mass. The female's habit of stirring the eggs with her walking legs may provide some opportunity for fungus spores to get into the interior, for in a few cases infection was found at a distance of 5 or 6 mm. down the filament. In only one sponge were infected eggs ever observed at the base of a filament and this filament measured but 13 mm. in length and was located at the outer end of the pleopod. Occurrence of infection inside the sponge, although uncommon, nevertheless provides positive evidence that conditions below the surface of a sponge are suitable for fungus growth. Hence, it seems that the outer eggs must act as a buffer providing a surface for attachment of organisms and a filter for the water that penetrates the sponge.

An infected area of a sponge increases rapidly in diameter while its penetration is much slower. A filament in such an area usually has all the eggs diseased for a length of from one to three millimeters at the distal end. Under the microscope the infection can be seen in various stages. In very heavy infections, the most distal eggs have had their nutrient material exhausted by the mycelium and resting cells have formed while the egg membranes may have started to disintegrate. Adjacent eggs to these have become very opaque and dwarfed and possess external hyphae and sporangia. The diseased eggs which are lowest on the strand are in the earlier

stages of infection with only one or two empty spore cells on the outside; the internal mycelium is still developing, and very few or no external hyphae are visible.

In this study, examinations were made on disintegrating eggs at the distal ends of the filaments. If the disease destroys eggs rapidly it seems that many of the filaments would lose the cuticular covering which supports the eggs. However, no filaments were found where infection had progressed this far.

A small percentage of eggs destroyed by infection. In laboratory hatching experiments, uninfected egg-bearing filaments yield about a 90 per cent hatch (Sandoz and Rogers, 1944). Numerous empty egg cases observed on sponges removed from spawning grounds also indicate a high hatching percentage of uninfected egg masses in their natural environment. In the case of infected sponges, diseased eggs do not hatch but among the uninfected eggs on the same sponge it was found that the hatching percentage seems to remain high. Several sponges were examined which showed a very heavy peripheral infection beneath which the interior eggs were nearly all hatched out.

On an average-sized sponge of 2,000,000 eggs, about 10 eggs are distributed per millimeter of filament. The average length of all the filaments of a sponge is approximately 12 mm. In very heavy degrees of infection, if the distal 3 mm. of all the filaments were infected, there would be about a 25 per cent infection of the total number of eggs in the sponge. Of the total 2,000,000 eggs, 75 per cent or 1,500,000 eggs are in the interior and do not become infected but complete embryonic development and hatch normally. Moreover, a very heavy degree of infection occurs in slightly less than 25 per cent of the sponges; therefore it seems unlikely that *L. callinectes* can be regarded as a factor in the fluctuations of crab populations.

Older sponges more heavily infected. Samples of sponges in any age group show all degrees of infection; in view of which eggs must be susceptible to fungus spores throughout their developmental period. A large number of moderately infected egg masses with diseased patches on opposite sides, or on separate pleopods, indicate that a mass of eggs may be attacked by many spores simultaneously. In most of the sponges where slight infection was present, diseased eggs were found widely distributed over the periphery.

Figure 6 shows the incidence of infection by *L. callinectes* among sponges of different age groups throughout the summer of 1944. Less than 50 per cent of the yellow sponges showed infection while both brown and black sponges had a higher percentage of infection. This increase of infection in brown and black sponges is believed to be due to the eggs being older and, hence, exposed to infection for a longer time.

In examining the samples, consideration was given to the amount of infection present on each sponge. The various degrees of infection remained in almost equal proportions throughout the summer, thus indicating a fairly regular cycle for the parasite as regards the continuous infection of sponge crabs during the spawning season.

Zoeal larvae may become infected. In the laboratory, when infected eggs were present in the filaments, zoeae which had hatched normally often showed evidence of the fungus. It is believed that infection could not have occurred before hatching because the mycelium within 48 hours is able to fill an entire egg (Couch, 1942), and in all probability embryonic development would become disturbed within a few

hours at most after penetration of the spore. The zoeae possess a very thin exoskeleton quite similar in appearance and thickness to the egg membranes which spores penetrate easily. It is more probable that zoeal infection occurs following a normal hatching of the egg. None of the larvae taken from plankton have ever been observed with fungus infection. Infected zoeae have been seen only in laboratory hatching pans, where the larvae must swim about in spore infested water. Under natural conditions larvae hatch from the sponge of the mother crab as she rests on the bottom in warm shallow water. They are positively phototropic and

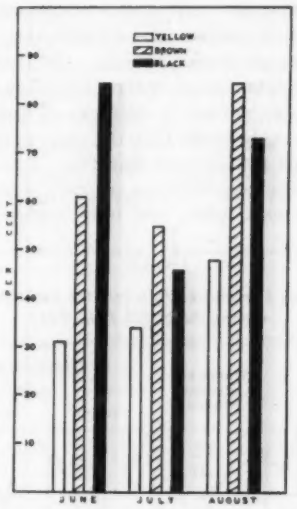


FIGURE 6. Percentage of yellow, brown, and black sponges infected by *Lagenidium callinectes*. Hampton Roads-Lynnhaven area. 1944.

begin to move toward the surface. In this way the young swim away from the old sponge where infection may have contaminated the surrounding water with many motile spores. When the fungus invades the zoeae, the larvae soon weaken and become unable to swim. If such infection does occur in nature, this would explain the absence of diseased individuals from the plankton samples that we have studied.

Transmission of infection under experimental conditions. Laboratory cultures showed that transmission of infection from one egg to another is extremely rapid. Often an entire pan of eggs was destroyed by disease in three or four days, even when the first day showed very few infected eggs. In aquaria, healthy egg-bearing crabs quickly became infected when diseased crabs were introduced. In one case, water from an aquarium inhabited by a single infected female was used in a hatching pan which contained only normal eggs. Within two or three days *L. callinectes* was seen and a majority of the eggs soon became infected. In other experiments in which diseased and normal eggs were placed at opposite ends of a pan, the fungus was observed to infect the normal eggs after about two days.

Infected sponges which were suspended in the York River failed to hatch. During the experiment the number of infected eggs increased while the uninfected ones under the same conditions hatched normally, the zoeal larvae escaped and left behind their empty, transparent egg cases.

For experimental purposes normal eggs were usually selected from the Seaford catches where diseased crabs were seldom observed. There is no record of infection in the York River; consequently the chances of fungus having been introduced from the Seaford or York River waters are slight. Examination of controls never showed fungus growth.

Factors affecting the fungus. Laboratory experiments have demonstrated a wide salinity tolerance for this fungus. In all salinities, from 5 to 30 p.p.t., hyphal growth and spore formation proceeded rapidly. In fresh water during a two day period there was some development of external hyphae and a few small abnormal sporangia. During a two day period in salinities of approximately 15, 20, 25, and 30 p.p.t. there was such heavy growth that the eggs appeared to be enveloped in a fine white down. New eggs also became infected. In a salinity of 20 p.p.t. where the parasitic growth was extremely heavy, a typically infected crab egg was observed with seven sporangia, four exit tubes, and four hyphae, all visible from one side.

TABLE I

Percentage of sponges from Chesapeake Bay infected by Lagenidium callinectes Couch during the period 1942–1944

Location	Year	Number of sponges examined	Distribution of infection			Percentage of sponge infection
			Yellow	Brown	Black	
Lower Bay	1942	82	1	13	19	40
Lynnhaven Roads	1943	30	3	9	4	53
Lynnhaven Roads	1944	393	78	104	60	62
Lynnhaven River	1943	12	6	1	0	58
Lynnhaven River	1944	37	13	8	0	57
Hampton Roads	1943	15	1	8	4	87
Hampton Roads	1944	136	20	19	16	40
Ballards Marsh	1944	11	0	0	0	0
Seaford	1943	76	0	0	1	1
Seaford	1944	254	6	4	4	5.5
York River (at Yorktown)	1944	63	0	0	0	0
Rappahannock River	1943	6	0	0	0	0

L. callinectes can withstand sudden changes in salinity. The sponge crab used in this experiment was taken from Lynnhaven where the salinity is about 27 p.p.t. She was carried in a moist basket to the laboratory; eggs were cut from the sponge and placed in York River water (salinity 20 p.p.t.) for about an hour. When the salinity series was set up, the sponge filaments were transferred directly to pond water and salinities of 5, 10, 15, 20, 25, and 30 p.p.t. In no case except pond water was there apparent retardation in fungus growth. Development in salinity as low as 5 p.p.t. suggests that it may be possible for *L. callinectes* to become conditioned to very brackish water.

Low temperatures were observed to retard fungus development somewhat. This was first noticed in hatching experiments in 1942. When diseased eggs were placed in the refrigerator (15 to 16 degrees C.) fungus development and spore formation were delayed. This temperature, however, did not prevent sporulation and the spores continued to swim about, but their movement was sluggish.

Distribution of Lagenidium callinectes in Chesapeake Bay. Extensive samples of sponges collected during 1943–44 have indicated that *L. callinectes* is quite common in waters extending from Hampton Roads to Cape Henry (Fig. 1). However, the disease is not confined to these open areas. Samples from neighboring places also revealed the existence of fungus in inlets of the region. Samples from several miles up the Lynnhaven River showed a high percentage of fungus occurrence. In August, 1943, a sample from this river showed a 58 per cent infection; in July, 1944, another sample showed a 57 per cent infection (Table 1). In 1942, infected sponges were found in Pagan's Creek, a tributary of the James River. However, in August, 1944, a sample from Ballard's Marsh at the James River Bridge was not infected. In July, 1941, fungus was observed in a sample from Buckroe Beach, which represents the northerly limit of heavily infested waters.

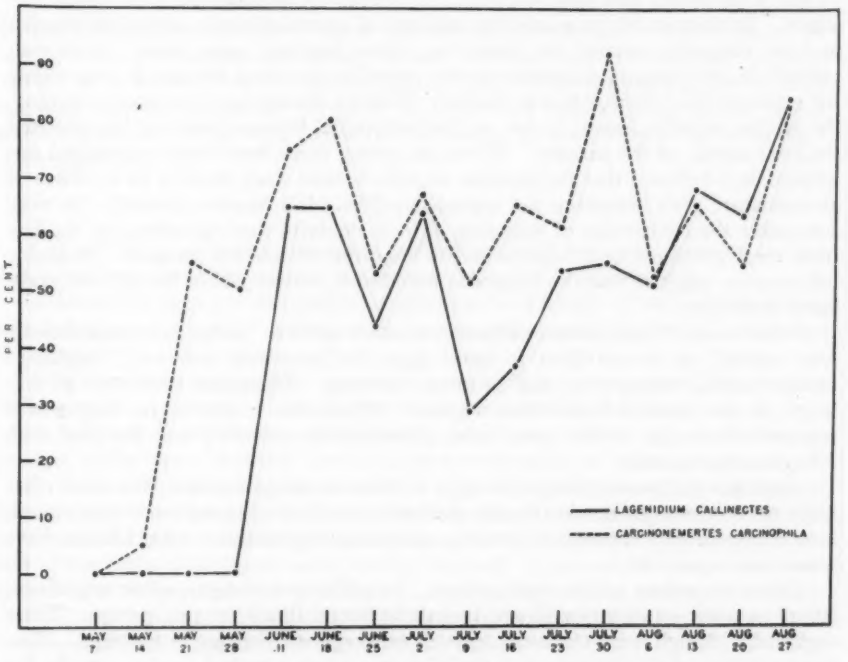


FIGURE 7. Percentage of blue crab sponges infected by the fungus *Lagenidium callinectes* Couch and by the nemertine *Carcinonemertes carcinophila* Kölliker during the summer 1944. Hampton Roads-Lynnhaven area. (Dates indicate first day of the week during which collections were made.)

In 1944, sponge crabs first appeared in commercial catches during the second week in May. The fungus was not present until a month later, the first record being taken from a sample collected on June 11 in which 13 out of 20 sponges were infected (Fig. 7). There seems to have been a simultaneous appearance of the fungus in both the Hampton Roads and Lynnhaven areas. This would indicate that the organism is well distributed throughout the region, spends a quiescent winter, and becomes active as soon as favorable conditions return. Egg-bearing crabs disappeared soon after August 31, 1944, until which time the fungus was present in more than 50 per cent of the specimens with a small increase during the last of August.

Samples from other regions of Chesapeake Bay have been examined. Throughout the Seaford area infection is uncommon. Several samples taken during June and August showed a 2 to 3 per cent infection. In one sample taken off Egg Island Bar at the mouth of Back River infection occurred in 45 per cent of the sponges. For this region the figure is high; however, Egg Island Bar is located in waters not far distant from Hampton Roads and the sanctuary where there is infection. In the York River at Yorktown, *L. callinectes* has not been found. One or two infected sponges have been taken from Mobjack Bay, Poquoson River, and the mouths of the York River and Back Creek. The degree of infection in most cases was slight. In these waters however, the majority of spawning crabs are yellow in color and are migrating toward the lower bay where hatching takes place. It is concluded that the general migration to the capes of spawning females is responsible for retaining the infection in this locality. When a female has completed spawning, the fungus probably ceases to live on that individual because hatching has depleted the food supply of the parasite. When the young crabs begin their northward migration, it is believed that the parasite remains behind since there is no evidence of an immature crab harboring the organism. The adult females probably die very soon after the completion of spawning so it is doubtful that spreading up the bay from the Lynnhaven area could occur by migration of infected females. Available information suggests that the fungus is localized in waters where female blue crabs hatch their eggs.

Occurrence of Lagenidium callinectes in other species. Laboratory experiments were carried out in an effort to infect eggs of *Pinnotheres ostreum, Neopanope texana, Libinia emarginata,* and *Sesarma cinereum.* The crabs used were all collected in the Seaford-York River region. Within two to five days, fungus was transmitted to eggs of the oyster crab (*Pinnotheres ostreum*) and the mud crab (*Neopanope texiana*).

Attempts to transmit fungus to eggs of *Libinia emarginata* and *Sesarmas cinereum* were unsuccessful, even though the latter remained alive for more than a week in the laboratory. However, previous hatching experiments with Libinia have never been successful.

Other organisms on the crab sponge. In addition to fungus, other organisms, either parasitic or commensal, are frequently found living on the sponge. These organisms, though quite common, seem to do very little damage to the eggs. Protozoan forms of *Carchesium* and *Ephelota* are often attached to the eggs in the peripheral portion of the sponge.

When fungus was first observed on the eggs of *Callinectes sapidus,* a hair-like growth longer than the diameter of a crab egg was noticed. Some eggs showed a

profuse development of such filaments which at first were confused with the parasitic fungus. However, the filamentous growth later was recognized to be a *Chlamydobacterium* (sp.).

In 1944, while conducting crab studies at this laboratory, Dr. Sewell H. Hopkins found the parasitic nemertine *Carcinonemertes carcinophila* Kölliker (compare Humes, 1942) to be very abundant on the gills of the blue crab. This worm was likewise observed embedded in the sponge where it deposited its own eggs in a case entwined around the filaments. In these observations it was noticed that the nemertine and the fungus frequently occurred together (Fig. 7). The factors governing infection by *L. callinectes* and *C. carcinophila* appear to be quite similar, since the results show a corresponding periodic fluctuation of the two.

Significance of Lagenidium callinectes. From this discussion, *Lagenidium callinectes* has been found to be a peripheral parasite of the egg mass of the blue crab and the data obtained show that it is present in a large percentage of sponges (Table 1). When present, although it spreads rapidly among the peripheral eggs, penetration into the sponge is slow and rarely deep. Meanwhile, the healthy eggs of the interior, which in all cases represent at least three-fourths of the mass, continue their development and hatch normally.

This parasite, now evidently established within the spawning area, may possess the potential ability to destroy a great number of blue crab eggs. However, in the light of these observations, prevailing natural conditions seem to hold the fungus in check. It is known that the parasite has a fairly wide temperature and salinity tolerance, but the incubation period of the blue crab lasts only about two weeks which appears to be too brief a time for the fungus to work deeply into the center of an egg mass.

Summary

1. The fungus parasite *Lagenidium callinectes* Couch has been observed to be a peripheral parasite of egg masses of the blue crabs of Chesapeake Bay.

2. Blue crab eggs are susceptible to infection in all stages of their development. Infected areas of a sponge are brown or gray in appearance, depending on the age of the eggs.

3. While the fungus spreads rapidly over the surface of the sponge, it penetrates the egg mass very slowly. Usually the depth of infection is not over 3 mm.

4. Infection is heavier in older sponges which are brown and black than in younger yellow ones, probably due to the longer exposure of older sponges.

5. Peripheral infection does not seem to retard the development of crab eggs in the interior of the sponge, which far outnumber the peripheral eggs. Not over 25 per cent of the eggs of a heavily diseased sponge are infected and only about 14 per cent of the crabs were found to be heavily infected. However, it was not unusual to find 80 or 90 per cent of the crabs in a sample to have some degree of infection.

6. Under laboratory conditions, transmission of infection from egg to egg of the same and different blue crabs is unexpectedly rapid.

7. Development of the fungus was observed to be abnormal in fresh pond water. In salinities from 5 to 30 p.p.t. development proceeded rapidly and indicated a strong tolerance of changes in salt concentration.

8. Frequently occurring on the peripheral eggs with *Lagenidium callinectes* are *Carchesium* sp., *Ephelota* sp., and *Chlamydobacterium* sp. *Carcinonemertes carcinophila* Kölliker is present and shows periodic fluctuations similar to the fungus.

9. Eggs of the oyster crab and the mud crab became infected with *L. callinectes* under laboratory conditions.

10. The Hampton Roads-Lynnhaven waters is the area in Chesapeake Bay where *L. callinectes* is most common. Only slight infection was observed north of Buckroe Beach.

LITERATURE CITED

CHURCHILL, E. P., 1919. Life history of the blue crab. Bull. U. S. Bureau of Fisheries, 36, Document No. 870, 1919, pp. 96–123, Washington, D. C.

COUCH, J. N., 1942. A new fungus on crab eggs. *J. Elisha Mitchell Scientific Society*, **58** (2): 158–162.

HOPKINS, S. H., 1944. The external morphology of the third and fourth zoeal stages of the blue crab, *Callinectes sapidus* Rathbun. *Biological Bull.*, **87** (2): 145–152.

HUMES, A. G., 1942. Morphology, taxonomy, bionomics of the Nemertine genus Carcinonemertes. Ill. Biol. Monographs, 18, No. 4, 105 pages.

LOCHHEAD, M. S., AND C. L. NEWCOMBE, 1942. Methods of hatching eggs of the blue crab. *Va. Jour. of Sci.*, **3**: 76–86.

SANDOZ, M. D., AND R. ROGERS, 1944. The effect of environmental factors on hatching, molting, and survival of zoeal larvae of the blue crab, *Callinectes sapidus* Rathbun. *Ecology*, **25**: 216–228.

SANDOZ, M. D., R. ROGERS, AND C. L. NEWCOMBE, 1944. Fungus infection of eggs of the blue crab, *Callinectes sapidus* Rathbun. *Science*, **99**: 124.

FACTORS INFLUENCING MOLTING AND THE SEXUAL CYCLES IN THE CRAYFISH [1]

HAROLD H. SCUDAMORE

Department of Zoology, Northwestern University

Introduction

There are two interesting phenomena associated with the periodic molting and the cycles of sexual functioning in crayfishes that have not been studied in detail. One of these interesting events is the delay in the spring molt of egg-carrying females until after the eggs hatch and the young crayfish leave the female; the other concerns the changes in the secondary sex characters in the male crayfish at the time of molt (Scudamore, 1942b). This investigation describes some of the factors influencing these two phenomena in the life cycle of the crayfish.

The delay in the spring molt of egg-carrying females has been observed by Van Deventer (1937), Tack (1941) and others. According to Tack (1941) the spring molt of males and non-reproducing females of the crayfish, *Cambarus immunis,* occurs about the middle of April in south central New York with most of the crayfish molting within the period of a few weeks. However, the females, which are carrying eggs at this time, do not molt until five or six weeks later. The reproducing females deposit their eggs in the fall shortly after mating and carry their eggs, attached to their abdominal pleopods, all winter. The eggs hatch about mid-May and the young remain dependent upon the female for a week or longer, while undergoing their first two molts. The egg-bearing female molts a few days after the young leave her pleopods.

The spring-molting period of males and non-reproducing females of the crayfish, *Cambarus propinquus,* also begins about the middle of April in central Illinois and lasts about three weeks (Van Deventer, 1937). However, the reproducing females do not deposit their eggs until early April. The eggs hatch about the middle of May and the young remain dependent for approximately another week. The egg-bearing females do not molt until late May or early June which is at least three weeks after the male spring-molting period.

This delay in molt of egg-bearing females is a protective adaptation, because molting earlier would result in the death of all the embryos. The mechanism producing this lag in the spring molt of ovigerous females has not been explained adequately. However, Hess (1941) reported a delay in molting of the seeded female shrimp, *Crangon armillatus,* as compared to non-seeded females. He also observed that removal of the embryos from seeded females shortened the period between molts and concluded that the factor, which inhibited molting, was apparently dependent upon attachment of the embryos to the female.

[1] The author wishes to acknowledge the constructive criticisms and encouragement offered by Dr. Frank A. Brown, Jr., during the course of this investigation and to express his sincere appreciation to Dr. C. L. Turner for the use of unpublished field data.

The male secondary sex characters studied were the first pair of abdominal pleopods which are modified as gonopods for the transfer of spermatozoa to the annulus ventralis of the female during copulation. These gonopods have been described sufficiently by Turner (1926), Van Deventer (1937), Tack (1941) and others. Male crayfish are classified as Form I, II or "juvenile" on the basis of the morphology and function of the gonopods. Mature males with sexually-functioning gonopods are designated as Form I, and those with non-functioning gonopods, as Form II; immature males with non-functioning gonopods are classified as "juvenile." Most mature males change from Form I to Form II at the spring molt and revert to Form I during the summer molt in time to function during the fall mating season, remaining in Form I until the following spring. No satisfactory explanation of these changes in sexual form has been noted in the literature.

It is possible that sex hormones are involved in the delay of the spring molt of egg-carrying females and in the changes of sexual form of the male gonopods at the time of molting. However, there is no conclusive evidence for the presence of sex hormones in the crustaceans. As pointed out by Brown (1944) most of the proof for the presence of sex hormones is based on indirect results such as parasitic castration, radiation and regeneration experiments.

Materials and Methods

Most of the laboratory experiments and some field observations were made on the crayfish, *Cambarus immunis* Hagen; but a few observations were made on *C. propinquus* Girard. The stock animals were kept in lead-lined tanks supplied with running tap water and were offered chopped earthworm or liver as food. The experimental crayfish were placed in individual finger bowls which were frequently refilled with fresh tap water and maintained at room temperature.

The eyestalks were removed by excising through the basal membrane with a sharp, pointed scalpel and coagulating the open wound with an electric cautery to control hemorrhage. Evidence of an approaching molt was obtained by sacrificing an animal and examining the anterior wall of the stomach for the presence of gastroliths. The pair of gastroliths were dried in an oven at 100° C. for 24 hours and weighed to determine gastrolith size. The carapace lengths were measured from the posterior margin of the cephalothorax to the tip of the rostrum.

The technique of inducing gastrolith formation and molting by removal of both eyestalks, developed by Brown and Cunningham (1939), Kyer (1942), Scudamore (1942a, 1947) and others, permitted (1) a study of secondary sex changes in male crayfish during winter molts induced by eyestalk removal as well as during normal spring and summer molts and (2) an investigation of the role of the eyestalks (sinus glands) upon molting in egg-carrying female crayfish.

Field Observations

Collections of *C. propinquus*,[2] made from a single locality during the spring and summer, illustrate the phenomena of the delay in the spring molt of egg-bearing females and the changes in sexual form of males (Table 1). On March 27th an ice jam had flooded a stream flat and, when the water receded, great numbers of

[2] From the unpublished records of collections made by Dr. C. L. Turner in 1921 from Turtle Creek, Rock County, Wisconsin.

crayfishes remained on the flat, either dead or in a dormant condition. All the males were Form I and had hard, calcareous exoskeletons. None of the females were bearing eggs. In this particular collection there were more females than males. According to Van Deventer (1937) males slightly exceed the females in number and, during the egg-bearing period, greatly out-number the females in the active population.

TABLE 1

Summary of molting and changes in the sexual cycles of the crayfish, C. propinquus, as observed in random field collections

Date	Total collected	Form I males		Form II males		Females		
		Number	Condition of exoskeleton	Number	Condition of exoskeleton	Number	Condition of exoskeleton	Number bearing eggs
March 27	270	109	Hard	0	—	161	Hard	0
April 15	76	68	Hard	0	—	8	Hard	7
April 24	156	124	Hard	0	—	32	Hard	26
May 1	48	46	Hard	0	—	2	Hard	2
May 13*	46	6	Hard	28	Soft	8	Hard	8
					Soft	4	Soft	0
June 3**	136	3	Hard	88	Medium	26	Hard	0
					Hard	19	Soft	0
July 6***	69	6	Soft	26	Hard	30	Soft	0
						34	Hard	0
Late July*** and August	Many	Many	Mostly hard	Very few	Hard	Many	Hard	None

* Spring molt of males and non-reproducing females.
** Spring molt of egg-bearing females.
*** Summer molt, both sexes.

On April 15th only eight females were secured in a collection of 76 crayfish which was made with a dip net without searching under stones. Seven of the females were bearing eggs upon their swimmerets. All the males were still Form I. Collections of April 24th and May 1st consisted chiefly of hard-shelled Form I males. The majority of the females were carrying eggs and were found hidden under stones. Apparently they had not moved from their hiding places or eaten for several days because an examination of their alimentary canals revealed no food. The few females not bearing eggs were moving around actively like the males.

By May 13th there were many cast-off exoskeletons lying in the margins of the shallow waters and practically all of these had come from Form I males but a few had come from non-reproducing females. Most of the males collected were soft-shelled and Form II, showing evidence of a recent molt. The four active females without eggs had molted recently. However, the eight females bearing eggs or young were concealed under rocks and had not molted.

Most of the males collected on June 3rd were Form II, indicating that they had completed the spring molt. The young crayfish, which up to this time had been clinging to the pleopods of the females, were in an advanced stage and many had

left the females altogether. Many of the females were soft-shelled as a result of a recent molt following escape of their young, and nearly all of the exuvia lying in the shallow water were those of females that had carried eggs.

By July 6th most of the egg-bearing females had molted and some of the males had completed their second or summer molt. In late July and early August most of the males collected were Form I, indicating that the summer molt was completed. Some of the females apparently had undergone a second molt at this time and become sexually functional. In contrast to this observation, Van Deventer (1937) reported that adult females, which have borne eggs during the spring, undergo only a single molt.

Although it is difficult to delineate accurately the various events because of the length of time between collections, certain generalizations may be made regarding the life cycle of *C. propinquus* in southern Wisconsin. (1) Reproducing females deposit their eggs in early April and carry their eggs until the middle of May. The eggs hatch about mid-May and the young remain attached to the female for several days. (2) The spring-molting period of males and non-reproducing females begins after May 1st with many animals molting by May 13th and the remainder before June 3rd. (3) The spring molt of egg-carrying females, which is delayed until after the young have left the female, occurs during the month of June or about three weeks after the male spring molt. (4) The second or summer molt of most mature males and at least some females takes place during July and early August. (5) Most mature males change from Form I to Form II during the spring molt and from Form II to Form I during the summer molt.

Molting in Egg-Carrying Females

In order to determine the role of attachment of the eggs to the swimmerets in delaying molt, a number of egg-bearing female crayfish, *C. propinquus,* were placed in a large aquarium, closely simulating the natural environment, during the spring-molting period of males and non-reproducing females. The eggs were then removed from one group of females. Both those with eggs attached and those with eggs removed were given access to an abundant food supply. The egg-carrying females remained in their hiding places beneath stones in the aquarium, waving the mass of eggs attached to their swimmerets but not feeding. On the other hand, the crayfish, from which the eggs had been removed, soon began to move about freely, fed actively and molted within one or two weeks.

In experiments performed during the winter of 1941–42, twenty normal egg-carrying female crayfish, *C. immunis,* were placed in individual finger bowls and all of the eggs removed from the pleopods of ten of them; both eyestalks were extirpated from another group of twenty egg-bearing females, and the eggs removed from ten of these animals. The crayfish from each of the four groups were sacrificed (or died) and examined for the presence of gastroliths at various intervals of time. One eyestalkless crayfish in each group died of operative injury before sufficient time had elapsed for gastroliths to form (Scudamore, 1947) and so were not included in tabulating the results.

Only three of the normal egg-carrying crayfish were found to contain very small gastroliths and none molted (Table 2), even though these animals were observed for a period nearly three times longer than the eyestalkless crayfish (Table 3). The

experimental period was long enough to permit gastrolith formation and molting when compared to normal pre-molt periods (Scudamore, 1947). Minute gastroliths have been observed in other normal crayfish during the winter, but their exact significance is not-known. There was no essential difference in the results whether the eggs were removed or not. This experiment demonstrated that the mere removal of the eggs from the normal crayfish does not induce molting in a non-molting season.

TABLE 2

Influence of removing the eggs from the pleopods of normal egg-carrying female crayfish, C. immunis, upon gastrolith formation and molting from December to February

	Carrying eggs	Eggs removed
Number of animals	10	10
Average duration of experiment (days)	34.5	39.8
Number with gastroliths	2	1
Average weight of both gastroliths (mgm.)	0.25	0.06
Number molted	0	0
Average carapace length (mm.)	30.6	28.5

TABLE 3

Effect of bilateral eyestalk extirpation upon gastrolith formation and molting of egg-carrying female crayfish, C. immunis, from December to February

	Carrying eggs	Eggs removed
Number of animals	9	9
Average duration of experiment (days)	12.7	15.2
Number with gastroliths	9	9
Average weight of both gastroliths (mgm.)	44.80	54.19
Number molted	1	2
Average carapace length (mm.)	28.6	27.8

All of the eyestalkless crayfish had formed large gastroliths in 5–19 days after eyestalk removal and three of them had molted between 15 and 17 days after operation, even though winter is normally not a molting season (Table 3). The removal of the eyestalks resulted in gastrolith formation or molting whether the eggs were present or not. Although there was considerable individual variation, there was no significant difference in the rate of gastrolith formation in the two groups of eyestalkless crayfish (Table 4). The inhibition of molt seemed to be dependent upon both the presence of the eyestalks (sinus glands) and the attachment of the eggs to the pleopods of the female and not simply the attachment of the embryos to the female as concluded by Hess (1941).

Although the evidence suggests that the sinus gland molt-inhibiting hormone is responsible for the delay in the spring molt of egg-bearing female crayfish, there are a number of possible factors that may operate in maintaining the sinus gland activity until after the eggs hatch and the young leave the female, namely: hormonal, metabolic or nervous factors.

A female sex hormone elaborated in the ovaries or other tissues may cooperate with the sinus gland activity in the delay of molt. However, the lack of histological or experimental evidence for such glandular tissue (Brown, 1944) weakens this hypothesis. In this connection Turner (1935) reached the conclusion, on the basis

of morphological studies, that the complete development of the annulus ventralis, a female secondary sex character, depends upon some ovarian tissue and the total absence of any testicular tissue. Yonge (1937) reported a cycle of histological changes in the oviducal epithelium and of secretion from the "cement" glands of the pleopods associated with egg-laying and attachment of the eggs to the pleopods of the lobster and suggested that, in the absence of nervous connections to the epithelium or the glands, the cycle of changes seemed to be controlled by hormones.

TABLE 4

Rate of gastrolith formation in eyestalkless egg-carrying female crayfish, C. immunis, during the winter

Day after eyestalk removal	Carrying eggs		Eggs removed	
	Length of carapace (mm.)	Weight of gastroliths (mgm.)	Length of carapace (mm.)	Weight of gastroliths (mgm.)
13	28.7	19.6	28.7	10.3
13	28.0	67.9	28.3	81.0
13	——	——	27.5	21.1
15	29.8	22.7	26.8	49.8
15	29.8	73.8	29.5	97.2*
16	23.2	40.2*	28.8	84.2
16	——	——	29.6	33.8
17	——	——	26.4	50.1*
19	31.5	69.7	25.0	60.2

* Indicates that animal molted.

The annual cycle of metabolic changes, which normally may initiate molting directly or through inhibition of the sinus glands, may be delayed in the egg-carrying females. The importance of metabolic factors is emphasized by the observation that egg-bearing female crayfish are largely inactive, hiding under stones and not feeding freely until after the young leave the pleopods. Furthermore, the egg-bearing females become active, feed and molt within a short period of time after the eggs are removed artificially during the male spring-molting period.

Finally, prolongation of the molt-inhibiting action of the sinus glands by impulses over nerve-reflex pathways produced by the presence of the eggs on the pleopods may explain the delay in molt. Welsh (1941) has demonstrated morphologically an innervation of the sinus glands of the crayfish from the "brain." Moreover, the tracts followed within the central nervous system of the crayfish by sensory impulses from stimulation of proprioceptors and sensory hairs of the abdominal pleopods were traced functionally by Prosser (1935), confirming the neurone paths first described histologically by Retzius (1890). These observations suggest the nervous pathways which may be involved in the reflex stimulation of the sinus glands. However, the fact that removal of only the eggs and not the eyestalks did not initiate gastrolith formation in the winter even in the warmth of the laboratory, suggests that some factor or factors other than possible pleopod-sinus-gland reflexes are involved.

Some combination of hormonal, metabolic and nervous factors seems like the most plausible explanation of this phenomenon. It is apparent that further experi-

mentation is needed to establish the exact mechanism involved in the delay of the spring molt of egg-carrying females.

Molting and the Sexual Cycle of Males

The cycle of changes in sexual form of the first pair of abdominal appendages is illustrated by observations on molting in a single male crayfish, *C. immunis*, between April and September. This crayfish changed from Form I to Form II at the first or spring molt on May 15th and changed from Form II to Form I at the second or summer molt on July 15th.

In order to study the changes in sexual form of crayfish during the fall and winter, observations were made of changes in sexual form following molting of mature crayfish, *C. immunis*, induced by bilateral eyestalk extirpation and these changes were compared with those occurring at normal spring and summer molts. As shown in Table 5, all the crayfish became Form II after the artificially induced winter molts whether they were Form I or II before molt. The normal crayfishes changed from Form I to Form II at the spring molt and from Form II to Form I at the summer molt. Enough Form II males for this winter experiment were obtained by selection from a large number of animals, because most of the males were Form I.

Table 5

Changes in sexual form of mature male crayfish, C. immunis, at the time of molt

Period	No. of animals	Original sexual form	Form after molt
Spring molt	12	I	II
Summer molt	12	II	I
Winter molt, induced by eyestalk removal (November to March)	12	I	II
	10	II	II

While studying spermatogenesis of the crayfish, Fasten (1914) found a seasonal variation in the size of the testes and in germ cell proliferation. The testes commenced active proliferation and increased in size in June, reached greatest activity and size in July, remained large in August with their tubules filled with spermatozoa, decreased in size in September, and remained small until the following summer. This seasonal cycle of changes in the testes of mature males is illustrated in Figure 1 together with the duration of the spring- and summer-molting periods, the seasons during which Form I and Form II mature male crayfish predominate, and the time at which copulation occurs.

The period of greatest testis activity (July–August) coincides exactly with the summer-molting period when males change from Form II to Form I. Copulation ensues a few weeks later at a time when the males have Form I gonopods and the testis tubules are filled with spermatozoa. During spring molts and during winter molts induced by eyestalk removal, when the testis size and spermatogenic activity are at a minimum, the male crayfish changes to Form II.

These observations offer circumstantial evidence in support of an hypothesis

that variations in the amount of a male sex hormone, produced in the testes or other body tissues, are responsible for the changes in sexual form of the male gonopods at the time of molt. The cyclical release of such a hormone could be influenced by other internal or by environmental factors. The greatest weakness of this hypothesis is the lack of conclusive histological or experimental evidence for the presence of secretory cells within the testes. On the basis of morphological studies of the crayfish, Turner (1935) has considered that the development of aberrant secondary sex characters is largely dependent upon genetic rather than hormonal factors. However, the seasonal changes from Form I to Form II and the reverse obviously are not controlled genetically, since they occur in a single individual.

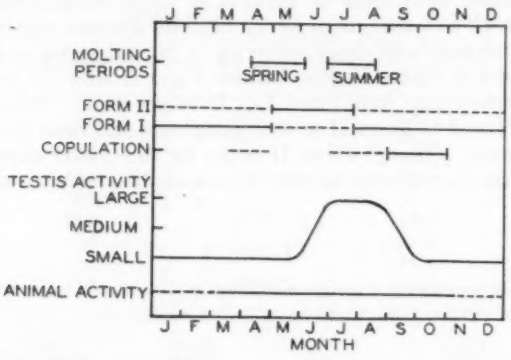


FIGURE 1. Diagram of certain phases in the life cycle of the mature male crayfish, *C. immunis*, demonstrating the relationship of molting periods, duration of and time of change to each male sexual form, period of copulation, periods of animal activity, and cycle of changes in testis size and spermatogenic activity. The curve of testis activity is based on the results of Fasten (1914). The solid lines (——) represent periods of predominant occurrence; broken lines (– – –), periods of occasional occurrence.

Proof for the existence of male, as well as of female, sex hormones must await histological evidence of secretory cells in the gonads or other tissues and the establishment of definite endocrine functions of these gland cells by surgical extirpation, implantation and injection of specific gland substances. However, observation and experimentation investigating the seasonal changes in the male gonopods represents a promising method for studying the problem of the existence of male hormones in crustaceans—a problem which is far from satisfactorily settled at this time.

SUMMARY

1. The phenomena of the delay in spring molt of egg-carrying females and of the changes in sexual form of males at the time of molt are described and illustrated by field observations on the crayfish, *C. propinquus*.

2. Removal of the eggs from the pleopods of egg-bearing females, *C. propinquus*, during the male spring-molting period results in an earlier onset of molting.

3. The delay in the spring molt of egg-carrying female crayfish, *C. immunis* and *C. propinquus*, is regulated by the action of the molt-inhibiting hormone of the sinus glands.

4. Various factors, that may operate to maintain the sinus gland activity until after the eggs hatch and the young leave the female, are discussed.

5. The changes in sexual form of male crayfish, *C. immunis*, at the time of spring and summer molts, and during winter molts induced by eyestalk extirpation are described.

6. Evidence is presented supporting an hypothesis that a male sex hormone, elaborated in the testes or other tissues, may regulate the cycle of changes in sexual form at the time of molt.

LITERATURE CITED

BROWN, F. A., JR., 1944. Hormones in the Crustacea: their sources and activities. *Quart. Rev. Biol.*, **19**: 32–46, 118–143.

BROWN, F. A., JR., AND O. CUNNINGHAM, 1939. Influence of the sinus gland of crustaceans on normal viability and ecdysis. *Biol. Bull.*, **77**: 104–114.

FASTEN, N., 1914. Spermatogenesis of American crayfish, Cambarus virilis and Cambarus immunis (?), with special reference to synapsis and the chromatoid bodies. *Jour. Morph.*, **25**: 587–649.

HESS, W. N., 1941. Factors influencing molting in the crustacean, Crangon armillatus. *Biol. Bull.*, **81**: 215–220.

KYER, D. L., 1942. The influence of the sinus glands on gastrolith formation in the crayfish. *Biol. Bull.*, **82**: 68–78.

PROSSER, C. L., 1935. Action potentials in the nervous system of the crayfish. III. Central responses to proprioceptive and tactile stimulation. *J. Comp. Neurol.*, **62**: 495–505.

RETZIUS, G., 1890. Zur Kenntnis des Nervensystems der Crustaceen. *Biol. Unters.*, **1**: 1–50.

SCUDAMORE, H. H., 1942a. Hormonal regulation of molting and some related phenomena in the crayfish, Cambarus immunis. *Anat. Rec.*, **84**: 514–515.

SCUDAMORE, H. H., 1942b. Hormonal influence on molting and the sexual cycle of the crayfish, Cambarus immunis. *Anat. Rec.*, **84**: 515–516.

SCUDAMORE, H. H., 1947. The influence of the sinus glands upon molting and associated changes in the crayfish. *Physiol. Zool.*, **20**: 187–208.

TACK, P. I., 1941. The life history and ecology of the crayfish, Cambarus immunis Hagen. *Amer. Mid. Nat.*, **25**: 420–446.

TURNER, C. L., 1926. The crayfishes of Ohio. *Ohio Biol. Surv. Bull.*, **3**: 145–195.

TURNER, C. L., 1935. The aberrant secondary sex characters of the crayfishes of the genus Cambarus. *Amer. Mid. Nat.*, **16**: 863–882.

VAN DEVENTER, W. C., 1937. Studies on the biology of the crayfish, Cambarus propinquus Girard. *Ill. Biol. Monogr.*, **15**: 7–57.

WELSH, J. H., 1941. The sinus glands and 24-hour cycles of retinal pigment migration in the crayfish. *Jour. Exp. Zool.*, **86**: 35–49.

YONGE, C. M., 1937. The nature and significance of the membranes surrounding the developing eggs of Homarus vulgaris and other Decapoda. *Proc. Zool. Soc. London*, **107A**: 499–517.

PROGRAM AND ABSTRACTS OF SCIENTIFIC PAPERS PRESENTED AT THE MARINE BIOLOGICAL LABORATORY, SUMMER OF 1948

JULY 6

Action pattern of crystalline muscle phosphorylase.[1] SHLOMO HESTRIN.[2]

Degradation of glycogen, amylopectin, and amylose by repeatedly recrystallized muscle phosphorylase was studied. The reaction was found to be:

polysaccharide + phosphate $\rightleftarrows$ limit-dextrin + glucose-1-phosphate

The limit-dextrin accounts for about 60 per cent of the weight of the parent polysaccharide in the case of glycogen and amylopectin, but must be a relatively minor reaction product in the case of amylose.

The phosphorylase limit-dextrin of glycogen was isolated and further characterized. The properties of this substance, in particular its ability to undergo a limited hydrolysis (24 per cent) by beta-amylase, are in accord with the view that it is derived from glycogen by a shortening of outer chains only, and that the final length of the shortened chains is probably three glucose units.

The phosphorylase limit-dextrin primes muscle phosphorylase and in suitable conditions can be shown to effect a shift in the equilibrium mediated by the enzyme. The findings thus further support Cori's theory that the primer is a stochiometric participant of the reaction.

Recrystallized muscle phosphorylase fails to degrade the beta amylase limit-dextrins of glycogen and amylopectin, and bacterial dextran. It is thus unable to cleave or by-pass an alpha 1–6 linkage and does not differ in this respect from potato phosphorylase as described by previous investigators. The enzyme may be regarded as a specific alpha 1–4 gluco-phosphorylase which acts on the 1–4 linkage only if the latter is terminal to a chain of sufficient length.

Crude muscle extract, in contrast to purified phosphorylase, converts glycogen and amylopectin almost quantitively to hexose phosphate. The factor in crude extract which mediates this effect is being studied further by Professor G. T. Cori and the author.

Vital Staining in ultraviolet and in white light combined. RUDOLPH KELLER.[3]

Living animals, such as salamander larvae, tadpoles, insect larvae, fish, daphnia, are first stained in the usual way by methylene blue, neutral red, alizarin, congo red or china blue and, afterwards, illuminated with luminescent dyes, such as primulin and aesculin, which make the daylight dyes visible in ultraviolet light.

With this method B. V. Pisha of this institution, using primulin, found that glands located in the distal end of the gut of daphnia showed a yellow greenish fluorescence. This, according to our former experiences, indicates the production of acid by the glands, which seems to neutralize the alkaline content of the gut.

In other experiments we stained with blue dyes such as aesculin (1 : 1000), positively charged in a biological medium, and administered after ten minutes, and a yellow dye uranin (1 : 10,000), also positively charged. We observed that the yellow dye rapidly displaced the blue one (which left the brood pouch of the daphnia at the neck) and the yellow uranin entered at the distal end of the pouch.

In further experiments we proceeded in the following way: To fish (Fundulus) in sea water in a vessel of 10 cc., three drops 1 per cent potassium ferrocyanide and two drops HCl 1/10 N

[1] This work was carried out in the Department of Biological Chemistry, Washington University School of Medicine, St. Louis, and was supported by a grant from the Corn Industries Research Foundation. The author is deeply indebted to Professor C. F. Cori for guidance in the conduct of the experiments.

[2] Hebrew University Travelling Fellow.

[3] Madison Foundation for Biochemical Research, New York.

were added. Later the animals were put into a solution of 1:800 thiazol yellow. The gills took up, first, in Prussian blue capillaries, only a little thiazol yellow. After killing the fish and adding new thiazol yellow, 1:800, to some gills on the slide, the space between some capillaries became strongly yellow greenish fluorescent, particularly in the neighborhood of injuries, while spaces between others appeared violet or scarcely stained.

The sulfhydryl metabolism of the beta cell and its relationship to the development of diabetes. ARNOLD LAZAROW.

Since glutathione protects rats from a diabetogenic dose of alloxan (Lazarow, *Proc. Soc. Exp. Biol. and Med.*, **61**: 441 (1946)), and since alloxan reacts with the sulfhydryl group of glutathione, and of protein, to give a new compound with an absorption spectra maximum at 305 mμ (Lazarow, Patterson, and Levey, *Science*, in press), it has been suggested that the glutathione, which is present in the pancreatic beta cell, normally serves to protect essential sulfhydryl enzymes from alloxan. It was further suggested that a low beta cell glutathione content could explain why alloxan is selective, but not specific, for beta cells.

It is estimated that the beta cells of man contain only 0.25 mgs. of glutathione [on the assumption that ½ per cent of the pancreatic weight (85 gms.) is islet tissue, and that the islet glutathione concentration is equal to that of the whole pancreas (60 mgs./100 gms.)]. Since insulin contains 12 per cent cystine, it is estimated that if all the cysteine contained in the glutathione of the beta cells of man could be incorporated into insulin, less than one milligram (19 units) of insulin would be formed. This is but a fraction of the daily insulin requirement of man.

Glutathione does not rapidly penetrate the cell membrane, for, on perfusion of liver, the glutathione is only slowly removed (Fabre and Simonnet, *C. R. de l'Acad. des Sci.*, **185**: 1628 (1927)). Following alloxan injection, the blood glutathione falls to near zero values, while the liver glutathione is only slightly affected. However, in spite of the large amount of glutathione in other tissues, the blood glutathione level has not returned to normal even after 6 hours (Leech and Bailey, *J. Biol. Chem.*, **157**: 525 (1945)). Thus cellular glutathione is not rapidly restored, and local depletions may take place. It is therefore postulated that the synthesis of insulin, in physiological amounts, may produce a local depletion in beta cell glutathione, and thereby render these cells more susceptible to alloxan or to other sulfhydryl inactivators, which may appear in the body.

Beta cell degeneration is also observed in (1) the pancreatic remnant following partial pancreatectomy, (2) following massive anterior pituitary hormone injections, and (3) after massive glucose injections. In all of these conditions, the beta cells are stimulated to an increased insulin production. It is further postulated that this increased insulin synthesis also sensitizes the beta cells to degeneration, because of a consequent local depletion in beta cell glutathione. If this theory of beta cell degeneration proves correct, then the glutathione metabolism of the beta cell will not only affect the etiology of alloxan, and other experimental diabetes, but it may also have an important bearing on the development of human diabetes.

JULY 13

A partial separation of the cytochromes of mammalian heart muscle. B. EICHEL, S. J. COOPERSTEIN AND W. W. WAINIO.

If successive amounts of sodium desoxycholate are added to an insoluble cytochrome complex preparation of mammalian heart muscle, the cytochromes can be partially separated. The desoxycholate is added at a concentration of 1 per cent and the undissolved residue in each instance is brought down by centrifugation at 20,000 × g for 1 hour. If 4 such successive fractions are prepared, the first fraction contains flavoprotein and cytochrome *c*, the second fraction contains cytochrome *c* and *b*, and the third and fourth fractions both contain cytochrome *b* and *oxidase*. However, fraction 3 has more *b* than *oxidase* and fraction 4 has more *oxidase* than *b*.

The absorption maxima in these preparations containing sodium desoxycholate were found at 416, 520, and 550 mμ for ferrocytochrome *c*, at 408 mμ for ferricytochrome *c*, at 429, 528 and 558 mμ for reduced cytochrome *b* and at 441 and 601 mμ for reduced cytochrome oxidase.

The identity of cytochrome *a* as a separate enzyme from cytochrome *oxidase* or a_3 (Keilin and Hartree terminology—*Proc. Roy. Soc. London,* **B127**: 167–91, 1939) is being investigated. The role of cytochrome *b* as a carrier for the dehydrogenases must be clarified with respect to its relation to the other cytochromes and to flavoprotein.

Alkaline phosphatase in demineralized mouse bones of different ages. ANITA ZORZOLI.

The general objective of this study was to determine the histochemical localization of the enzyme alkaline phosphatase in the tibia of normal mice during developmental life, during the period of growth and after growth of the bones had ceased. The bones were demineralized, without attendant enzyme inactivation, in a sodium acetate-acetic acid buffer at pH 4.55, sectioned and incubated with sodium glycerophosphate according to the method of Gomori.

Prior to the 15th day of gestation, the tibia was entirely cartilaginous and was completely devoid of enzyme. Phosphatase first appeared in the connective tissue surrounding a localized region of cartilage which was destined to become a center of ossification. Once the typical histological changes had begun, the enzyme appeared in the cartilage and was located in the nuclei, cytoplasm and to a slight extent in the matrix. Shortly later the bone salts became evident. It was interesting that their appearance was always preceded by that of the enzyme. With the spread of the processes of ossification the enzyme increased in amount and distribution.

In the early post-natal bones where rapid growth occurred, enzyme was prominent in the epiphyseal growth zone. The outermost cartilage cells of this region were small in size and were always phosphatase free. The adjoining cells, arranged in the form of columns parallel to the long axis of the bone, contained enzyme which increased in concentration with proximity to the hypertrophic zone. In the hypertrophic zone which was one of great activity, phosphatase occurred in both cells and cartilage matrix. It also appeared faintly in the calcified spicules and the newly formed bone. The osteoblasts were strongly phosphatase positive while osteoclasts were never observed to contain enzyme.

With increasing age the growth processes declined and the number of enzyme-containing cells of the hypertrophic zone decreased while phosphatase-free matrix increased. This change was already evident at 5½ months of age and by 13 months only a few scattered cells remained.

The order of amino acids in silk: an application of isotopic derivative technic. MILTON LEVY AND EVELYN SLOBODIANSKY.[1]

The principles described by Keston, Udenfriend and Cannan (*J. Am. Chem. Soc.* **68**: 390, 1946) are applicable to complex mixtures of amino acids, dipeptides and higher peptides as present in partial hydrolysates of silk. We have estimated the peptides of alanine (A) and glycine (G) in hydrolysates produced by the action of concentrated HCl at 39°. Thus, in a 48 hour hydrolysate the per cent of the total nitrogen in each form was: G, 12.9 per cent; A, 10.5 per cent; AG, 27 per cent; GA, 8.3 per cent; and GG, 1.8 per cent. Random arrangement of the 42.3 per cent G and 28.2 per cent A in our sample would have led to a maximum of 12.3 per cent AG. The arrangement cannot therefore be random. It is suggested that a unit of silk structure may be –G–X–A–G–A–G–X–. In this structure two AG's are possible for each GA. No GG is possible. X stands for any other amino acid. Further analysis of complete hydrolysates indicates in per cent of the total nitrogen the following amino acids: Serine, 9.24 per cent; glutamic acid, 1.07 per cent; aspartic acid, 1.54 per cent; hydroxyproline, 0.05 per cent. These analyses were done using the isotope derivative technic with separation by paper chromatography (Keston, Udenfriend and Levy, *J. Am. Chem. Soc.*, **69**: 315, 1947). It is noted that G, A and Serine are in the ratio of 9,6,2 and that glutamic and aspartic are in the ratio of 2:3.

The radioactive isotopes used in this work were supplied by the Clinton Laboratories on Allocation from the U.S.A.E.C. The work was supported by the American Cancer Society on recommendation of the Committee on Growth of the National Research Council.

[1] Stanley Tausend Foundation Fellow.

JULY 20

On the specificity of cholinesterase. KLAS-BERTIL AUGUSTINSSON.[1]

It has been demonstrated by different groups of investigators that the cholinesterase activities of various sources are not identical. A hypothesis has been proposed that two types of acetylcholine splitting enzymes exist.

There is no doubt, however, that many of the differences among the cholinesterases of various tissues cannot be accounted for by the fact that two types exist. This has been demonstrated in experiments with enzyme preparations from tissues and body fluids of various animals, vertebrates as well as invertebrates. A full account of these experiments has recently been published (Augustinsson, *Acta physiol. Scand.*, **15**: Suppl. 52, 1948).

Cholinesterases are defined as esterases which split choline esters at a higher rate than other esters; the specificity is not an absolute one. These esterases are regarded as a family of related enzymes with widely divergent properties. The following classification is based on the activity-substrate concentration relationships for the enzymic hydrolysis of acetylcholine.

Group I is characterized by the inhibition of cholinesterase activity at high acetylcholine concentrations; optimum activity at about 3×10^{-3} M acetylcholine. This group includes the cholinesterases of the nervous system, muscles, electric organs (Nachmansohn), erythrocytes, *Helix* blood, snake venom (Zeller). The enzymes of Group II follow the Michaelis-Menten formulation, which means that their activities are maximal only at infinite substrate concentration. This group includes the choline-ester splitting esterases of certain sera (e.g., man, horse), dart sac (*Helix pomatia*), and pancreas (Mendel). The properties of the members within each group may then differ in certain other respects.

The optimum conditions in the hydrolysis of acetylcholine are *a priori* not identical with those prevailing in the hydrolysis of other esters. When, for instance, the substrate concentration is arbitrarily chosen in the enzymic hydrolysis of a choline ester or a non-choline ester, the optimum conditions are not the same as those of the hydrolysis of acetylcholine, the rate of reaction may be lower, the same, or even higher than that of the acetylcholine hydrolysis.

Effect of anticholinesterases on conduction. DAVID NACHMANSOHN.

In 1942 the theory was proposed that the release and removal of acetylcholine are intracellular processes necessary for the conduction of the nerve impulse. This idea was based on a great variety of facts obtained by the study of the enzymes connected with acetylcholine metabolism, and their correlation with the electrical manifestations in conduction.

If the rapid removal of acetylcholine is necessary for the propagation of the impulse, anticholinesterases should block conduction. This could be shown with eserine and other inhibitors of cholinesterase (*J. Neurophysiol.*, **9**: 9, 1946). In this case, inhibition of the enzyme and block of conduction are reversible.

Two years ago, this theory was assailed by several investigators based on observations with a new anticholinesterase, diisopropylfluorophosphate (DFP). This compound inhibits cholinesterase irreversibly. All the objections raised, however, and the apparent difficulties have been overcome, and the necessity of cholinesterase for conduction has been demonstrated conclusively.

Contrary to the original assumption, the irreversible inactivation of cholinesterase by DFP is not an immediate process but depends on a number of controllable factors. A striking parallelism has been established between the rate of irreversible abolition of conduction and that of irreversible inactivation of cholinesterase. This has been shown as a function of time as well as of temperature. The necessity of the enzyme for conduction has been established on a great variety of different types of nerves, and on striated muscle suggesting the same role of acetylcholine in all conductive mechanisms throughout the animal kingdom (*J. Neurophysiol.*, **10**: 11, 1947). In no way is it possible to dissociate cholinesterase activity and conduction. Claims to the contrary were based on the use of inadequate techniques (*J. Neurophysiol.*, **11**: 125, 1948). Having met the challenge, the theory emerged from these discussions stronger than before (*Johns Hopkins Bull.* in press).

[1] Biochemical Institute, University of Stockholm.

The ion permeability of the giant axon of squid. M. A. ROTHENBERG.

The difference in concentration of ions between the inside and the outside of nerve fibers has long been assumed to be of importance in conduction (Ostwald, Bernstein, and many others). Most of the evidence suggesting ion movements during the passage of the impulse across the active membrane has been, however, of an indirect nature (see Hodgkin, *J. Physiol.*, **106**: 341, 1947). The exchange of ions across the nerve membrane of the giant axon of squid has now been measured by the direct determination of radioactive Na^{24} and K^{42} in the axoplasm of these fibers. In all cases, the outer environment was artificial sea water prepared according to Pantin (*J. Exp. Biol.*, **11**: 11, 1934), and no alteration in the concentration of ion species was made.

The results appear to indicate that during rest the ions inside the fiber may be in dynamic equilibrium with the same ion species outside. Thus, within 20–30 minutes all of the Na^{24} inside the fiber (based on figures of Steinbach and Spiegelman, *J. Cell. and Comp. Physiol.*, **22**: 187, 1943) exchanges for Na^{24} in the sea water. In the case of K^{42}, only about 10 per cent of the total inside will exchange within the same period of time. A twofold increase in the K^{42} penetration rate could be obtained with a two-fold increase in the K^{42} concentration in the sea water.

Attempts were made at determining the temperature coefficients for the Na^{24} and K^{42} exchanges across the membrane. A ten degree difference in temperature of the bathing fluid (12° and 22° C. resp.) showed no marked alteration in the rates of exchange. This supports the assumption that at rest there are no important chemical reactions involved in this exchange other than a Donnan equilibrium.

Electrical activity of the nerve increased markedly the rate of Na^{24} penetration. This same overall effect could be obtained by the addition of anticholinesterases to the sea water. For example, the addition of diisopropylfluorophosphate increased the Na^{24} penetration by about 50 per cent, and decreased the K^{42} penetration by about 35 per cent. This suggests an increase in membrane permeability and may, when studied more closely, throw some light on the immediate function of acetylcholine and cholinesterase in nervous conduction.

Cocaine, unlike the anticholinesterases, had a very small effect on the membrane permeability.

JULY 27

The extraction of purified squid "visual purple." A. F. BLISS.

The photochemistry of the squid retina has been studied by methods which have been successfully applied to vertebrate retinas. The common vertebrate visual pigment, rhodopsin, is replaced in the squid by a photostable homolog, cephalopsin. This pigment has been extracted in a purified state by a combination of Saito's and Lythgoe's methods for rhodopsin. Squid retinas are homogenized with 40 per cent sucrose and centrifuged at a high speed. This treatment separates the heavier melanin from the lighter red segments which contain the visual pigment. The red rod layer floats to the top and after separation is hardened with buffer at pH 4.5. It is then extracted with a mild detergent, aqueous digitonin. A clear red extract is obtained, whose absorption spectrum closely resembles that of rhodopsin. The maximum due to cephalopsin is at about 495 mμ in the blue green, while that due to rhodopsin is at 502 mμ. Cephalopsin becomes photosensitive in the presence of five per cent formalin and bleaches almost completely when exposed to light of a 100-watt lamp at a distance of one foot for 20 minutes. It is also bleached by strong acid and base in the dark. The bleaching products are indicator yellow and retinene, precisely as obtained from bleached rhodopsin. Cephalopsin apparently does not bleach *in vivo*. The increased retinene released from illuminated retinas shaken in petrol ether reported by Wald is probably a secondary physical effect due to pigment migration which increases the effective diffusion surface of the illuminated retina. Pigment migration fails in aerated retinas kept one hour after excision in complete darkness whereas Therman has shown that such retinas actually are more sensitive to light than freshly excised retinas. Retinas prepared according to Therman's procedure exhibited a light: dark retinene ratio of 1.02 ± 0.8, which does not differ significantly from unity. A detailed account of the purification of cephalopsin will appear in the *Journal of Biological Chemistry*.

Biochemical and histochemical observations on the sexual dimorphism of mouse submaxillary glands. L. C. JUNQUEIRA, A. FAJER, M. RABINOVITCH AND L. FRANKENTAHL.

Protease and amylase activity is demonstrated in extracts of mouse submaxillary glands. The protease content varies sexually but the amylase content does not. In castrated adult mice steroid sexual hormones influence the protease content of the glands. Correlation of biochemical and histological data locates the site of protease production in the tubular portions of these glands, and amylase synthesis in the acinar regions. Evidence is presented for location of ribonucleoproteins in the basal regions of acinar and tubular cells, and for variations in its content with sexual variations in the tubular cells. The secretory granules of the tubular cells appear to be of protein nature, as they give a positive reaction for phenolic amino acids and for sulfhydrilated proteins. "Acid" phosphatase is evidenced in the apical portions of the tubular cells, and shows histochemical and biochemical sexual variations. "Alkaline" phosphatase is distributed more diffusely throughout the gland. Our data suggest, but do not establish, sexual variation of the activity of this enzyme. Mucoproteins are present in the cytoplasm of the amylase producing acinar cells.

pH *estimation in reconstituting pieces of Tubularia stems.* JAMES A. MILLER, JR.[1]

Phenol red, chlor phenol red, brom thymol blue, brom cresol purple and brom cresol green were injected into the coelenteron of Tubularia with the aid of a Chambers micromanipulator. Of these, phenol red proved the most satisfactory both because of low toxicity and appropriate range.

The pH of uninjured stems ranged between 7.8 and 8.0. Cutting, crushing, inserting a pipette or even bending the stem sharply caused the release of an acid of injury the concentration of which depended upon the degree of injury. Recovery of normal pH required one to fifteen minutes, depending also on extent of injury.

Reconstituting stems released acid metabolites which maintained the coelenteric fluid at 0.2 to 0.4 pH below that of the uninjured stem. The first morphological indications of hydranth reconstitution were accompanied by regions of increased acidity corresponding to the two rows of tentacles. Once formed, the tentacles remained acid (pH 6.8 to 7.0) as also did the ring of perisarc-secreting tissue just proximal to the hydranth. The pH of other parts could be increased by increasing the availability of oxygen. Stolon formation and growth was not accompanied by observable changes in pH.

When reconstitution was blocked by placing stems in glass tubes, acidity was increased in all parts equally. Ligatured stems, on the other hand, showed the normal pH and in some cases developed regions of increased acidity at the ends indicating partial activation. One should therefore guard against considering that all ligatured stems are under truly basal conditions.

These experiments demonstrate that acid metabolites are produced in the ectoderm and endoderm of Tubularia and are liberated into the coelenteron. If prevented from escaping they increase the acidity to a point which has been found to inhibit reconstitution when externally applied.

The genetic block to free oviposition in the chalcidoid wasp Melittobia sp.—C. P. W. WHITING AND BERTINA M. BLAUCH.

Melittobia females normally fail to oviposit freely unless mated. The very rare exceptions reported ("layers") produce large progenies (100 to 300) of haploid sons. From a stock derived from a layer, 100 virgin females were isolated. The test showed 15 layers and 85 non-layers, the latter producing not more than four sons each. Sons of the layers were crossed with their aunts. Of 150 daughters tested, 51 were layers. The trait is clear-cut with no intergrades. The genetic basis, however, is complex. Selection, inbreeding and crossing the descendents for seven generations showed layer females per fraternity varying from 0 to 100%. From sibling matings in one line, three fraternities totalled 6 layers, 355 non-layers (1.66% layers), three fraternities totalled 84 layers, 326 non-layers (20.49%), one fraternity totalled 73 layers, 77 non-

[1] Emory University.

layers (48.66%). It was noted that layer females have a shorter life than non-layers and that there is close association of the trait with sterility and short life of their sisters. One layer when self-crossed (mated to a haploid son) produced 27 layers, 31 non-layers and 29 dying sterile after ten days (33.33% sterile). Another produced 22 layers, 4 non-layers and 20 dying sterile (43.48% sterile). Four produced 46 layers, no non-layers and 93 dying sterile (66.91% sterile). One self-crossed layer produced 6 layers, 240 non-layers (sterile not recorded). The trait is evidently not a recessive. Of these 240 non-layers, 15 were self-crossed. Grouped according to increasing percentage of layers and arranged as layers/non-layers/dying sterile, there were two fraternities, 3/73/12, with 13.64% dying sterile, five, 25/189/34, with 13.71%, one, 7/29/14, with 28.00%, five, 58/125/72, with 28.23%, and two, 34/37/28, with 28.28%. It is indicated that more than two factors may be involved with complementary effect and that the group dying sterile is genetically related to the layers.

AUGUST 3

Inhibition of sea urchin egg cleavage by a series of substituted carbamates. IVOR CORNMAN.[1]

Urethane (ethyl carbamate) is both carcinogenic, inducing lung tumors in rats and mice, and carcinostatic, diminishing tumor growth in rats and mice and lowering wbc count in murine and human leukemia. To correlate these properties with efficiency as a mitotic poison, eggs of *Lytechinus* and *Tripneustes* were exposed to a series of carbamates with methyl, ethyl, propyl, butyl, amyl and phenyl replacing the ethyl on the carboxyl or hydrogens on the amino end of urethane. These were supplied by Dr. C. D. Larsen of the National Cancer Inst. Exposure was begun 10 minutes after fertilization. Delay and blocking of cleavage were compared quantitatively. In general, effectiveness in blocking cleavage increased with increase in M.W. up to 8–10 carbon atoms. Isopropyl N-phenyl carbamate, Ethyl N,N-di-*n*-butyl carbamate and ethyl N-phenyl carbamate were the most efficient, blocking cleavage at 0.5–0.6 m Molar, while urethane required 56 mM/L. A cyclic configuration was the most effective, then straight-chain, branched-chain (*iso*), and divided groups (N,N-dimethyl, etc.), in decreasing order of activity. This parallels the narcotic activity of carbamates, but is entirely divergent from efficiency of pulmonary adenoma induction or of tumor suppression. For both of these, urethane is reported as the most active of the carbamates tested. These remarkable influences on the neoplastic process appear not to devolve in any direct way from destruction of mitosis.

A nuclear precursor to ribo- and desoxyribonucleic acids. A. MARSHAK.[2]

Rats were given P^{32} Na_2HPO_4 intravenously and nuclei isolated from the liver three hours later. Incubation of the nuclei at 37° C. with ribonuclease, desoxyribonuclease and with no added enzyme showed that 90 per cent of the nuclear P^{32} was in nucleic and the remainder in lipid and acid soluble fractions. Very little or none of the P^{32} was in DNA in nuclei from normal liver (no mitosis) and also in nuclei from regenerating liver (rapid mitosis). Incubation at 0°–2° C. releases no nucleic acid from the nuclei, although at 37°, 80 per cent is removed indicating the presence of an enzyme capable of splitting nucleoprotein or nucleic acid. The digestion products are not dialyzable and therefore are nucleic acid and not nucleotide. The native enzyme thus differs from ribonuclease which splits off nucleotide. The specific activity of the digestion products is 13 times as great as that of the RNA of the cytoplasm. The material extracted from the nuclei by the method for extracting RNA has a specific activity almost as high as that of the nuclei. This fraction may therefore be identical with the P^{32}-containing nuclear substrate but both are very different in behavior from the RNA of the cytoplasm. Extraction of the nuclei for 24 hours with 1 M NaCl removes only 20 per cent of the P^{32} and the P^{32}-containing material so extracted is not precipitated on dilution to 0.14 M NaCl. The P^{32}-containing nuclear material thus differs in solubility from desoxyribonucleoprotein. On the basis of the orcinol and diphenylamine reactions the nuclear auto-digestion products contain "RNA" and an amount of "DNA" less than 1/10 of the "RNA." Analyses of uracil and thymine con-

[1] Sloan-Kettering Inst. for Cancer Research.

[2] New York University College of Medicine. Tuberculosis Control Division, United States Public Health Service.

tent by preparing the p-iodophenylsulfanile derivatives with radioactive iodine show a thymine content less than 1/10 that of the uracil. In saline, desoxyribonuclease increases the rate of splitting of the P^{32}-containing substrate but in carbonate buffer at pH 7.0 it reduces the rate, suggesting binding of substrate without splitting it. The nucleic acid in question thus appears to have some properties in common with both DNA and RNA. In both mitotic and non-mitotic nuclei the P^{32} appears first in this nucleic acid; later, in mitotic cells, the P^{32} is accumulated in DNA, while in non-mitotic cells it passes into RNA of the cytoplasm, the P^{32} always being associated with nitrogenous base and sugar. Since it contributes to the formation of DNA and RNA and also since it has properties in common with each this nucleic acid is considered to be their precursor.

These findings indicate that plasmagenes and other cytoplasmic constituents containing nucleic acid cannot be independent of nuclear activity. They also predict that cells may be found which contain no DNA. Strains of bacteria with no desoxyribose and with no thymine have been reported.

The effect of "stabilizing" and "unstabilizing" agents in relation to the metabolic mechanism supporting the resting potential of nerve.[1] ABRAHAM M. SHANES.[2]

Certain agents which block conduction (e.g. calcium, cocaine, procaine) are known to enhance the polarization of nerve fibers; others, which increase excitability (e.g. calcium precipitants, veratrine), have been observed to cause depolarization. The former have been designated as "stabilizing," the latter as "unstabilizing."

It is now possible to characterize these two groups of substances by another effect, viz., their ability to modify the rate with which anoxia leads to depolarization. Thus, cocaine and procaine, in concentrations far below those which affect respiration (e.g. 0.001%), delay the decline of the resting potential in frog nerve during oxygen lack. Associated with this is a delay in the development of inexcitability. Spider crab (*Libinia emarginata*) leg nerves also are rendered less sensitive to anoxia by procaine, but the concentration required (ca. 0.05%) causes depolarization in oxygen.

Veratrine, like calcium precipitants, in a concentration which causes negligible depolarization in oxygen (1:200,000 for frog sciatic nerve, 1:2 million for crab nerve), speeds the depolarization process during anoxia and, when depolarization has not been excessive, increases the potential rise upon return to oxygen.

The exactly antagonistic effects of the stabilizing and unstabilizing compounds with respect to excitability, resting potential, and anoxia sensitivity suggest the involvement of a single basic locus. An explanation in terms of potassium permeability is in keeping with the available literature on the effects of these substances on potassium leakage and impedance; this is supported further by (a) the reduced depolarizing action of KCl on frog muscles treated with cocaine and (b) the ability of cocaine and procaine (0.2%) to slow the swelling of muscles in a Ringer in which some of the sodium has been replaced by potassium.

AUGUST 10

The relative rate of penetration of the lower fatty acids into beef red cells. JAMES W. GREEN.

Since the work of Overton the lipid solubility theory has been widely held to account for the penetration of lipid soluble compounds into cells. This theory may be tested by a study of the penetration rates of the lower fatty acids into mammalian erythrocytes. When a weak acid penetrates a mammalian red cell the oxyhemoglobin within the cell dissociates to a new equilibrium, the level of which appears to depend upon the amount and dissociation constant of the acid which entered the cell and the oxygen tension of the environment. Taking advantage of this dissociation of oxyhemoglobin, a modification of the Hartridge-Roughton rapid mixing

[1] This investigation was supported in part by a research grant from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service, and from the American Philosophical Society.

[2] Georgetown University School of Medicine.

technique was used to measure spectrophotometrically the rate at which the lower, saturated, monocarboxylic acids penetrated beef red cells. The method is essentially chemical and does not depend upon volume changes of the cell. Dilute unbuffered red cell suspensions were mixed with dilute acid solutions made up in 1 per cent NaCl such that the pH of the final mixture was maintained in the range 4.2.

The relative order of increasing rate of penetration of the acids studied for beef cells was found to be: formic, acetic, propionic, caprylic, heptylic, caproic, butyric, valeric. However, the rates of penetration of caproic, valeric and butyric acids could not be separated statistically. The actual time to 50 per cent penetration was calculated and found to vary from 0.138 seconds for valeric acid to 5.04 seconds for formic acid. From formic through caproic acids the relative rates of penetration are in agreement with results obtained by other methods and are in accord with the Overton theory. Heptylic and caprylic acids, although more lipid soluble than lower homologues, penetrate beef red cells more slowly than acids of smaller molecular volume. For this reason it is suggested that these two acids are limited in their penetration by reason of their larger molecular volumes.

Hemolysis studies were made with these acids, using the Parpart Densimeter. The hemolysis curves obtained, taken as a measure of penetration of these acids, did not support the findings using the spectrophotometric technique. It is thought that the discrepancies were owing to the inability of the hemolytic technique to distinguish between osmotic and lytic hemolysis.

Osmotic hemolysis in hypertonic solutions.[1] F. R. HUNTER.[2]

It has previously been noted in this laboratory that chicken erythrocytes standing for 12–24 hours in heparinized plasma at 37° C. become altered. Using a photoelectric apparatus to measure volume changes of these cells when they are placed in a hypertonic solution consisting of 0.3 M glycerol in Ringer Locke, a greater deflection of the galvanometer is noted when older cells (those which have stood at 37° C. for several hours) are used, as compared with new cells (those in freshly drawn blood). The apparent volumes, as measured photoelectrically, of both new and old cells in Ringer Locke, 2X Ringer Locke and 2X Ringer Locke to which an equal volume of water has subsequently been added are what would be predicted on the basis of the swelling curves. Spectrophotometric measurements of the amount of hemolysis in these various solutions show that as the cells stand in heparinized plasma at 37° C. there is an increase in their "fragility" as indicated by a large amount (up to 46 per cent) of hemolysis when old cells shrink and swell, the equilibrium medium being Ringer Locke. This hemolysis is noted whether the volume changes involve the penetration of the glycerol molecule or whether the cells shrink in 2X Ringer Locke and then swell again as a consequence of dilution of the medium with water. Hematocrit measurements show an increase in volume (5–20 per cent) as the cells stand in heparinized plasma. These cells also lose potassium and gain sodium. These experiments emphasize further the extreme sensitiveness of erythrocytes to their environment.

Hippuric acid excretion in anxiety states. HAROLD PERSKY.

The liver function of human subjects was determined by a variety of standard tests employed by the clinician. Only the sodium benzoate tolerance test (intravenous) was significantly altered in patients suffering with anxiety states. Controls were normal persons and other psychoneurotics hospitalized under identical conditions to the anxiety group but showing little visible anxiety.

The experimental and control subjects were physically healthy adults between the ages of 16 and 58. They had no clinical indications of liver disease nor any previous history of liver disease. They were in good nutritional status as judged by clinical examination and by a three day nitrogen balance study performed just before the liver function tests. The subjects had good kidney function as indicated by a normal serum urea level and in some instances also, a normal urea clearance.

[1] This work was supported by grants from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service and the Faculty Research Fund, The University of Oklahoma.

[2] Department of Zoological Sciences, The University of Oklahoma, Norman.

The degree of anxiety was assessed by a variety of clinical and psychological tests with general agreement among them qualitatively for each subject. For quantitative correlation, a new method of scoring the Rorschach test for anxiety was employed.

The anxiety group excreted an abnormally high amount of hippuric acid after i.v. injection of sodium benzoate. This effect was not observed in either of the two control groups. The excessive amount of hippuric acid in some instances was even greater than the theoretical yield of hippuric acid obtainable from the injected benzoate. The excretion of hippuric acid was directly related to the degree of anxiety for the anxiety state group. The relation is significant statistically. There is no correlation for the control groups.

Following psychotherapy, the hippuric acid is significantly decreased when the anxiety is decreased. The psychotherapeutic techniques used were: electric shock, insulin shock and/or psychotherapy.

The elevated hippuric acid excretion in anxiety states is due to an elevated endogenous hippuric acid production in anxiety states over the control groups. This endogenous hippuric acid exceeds that of the control groups from two to ten times. The absolute amount is adequate to explain the supernormal values obtained when sodium benzoate is administered.

The endogenous hippuric acid probably is derived from phenylalanine metabolism. It is postulated that the excessive hippuric acid obtained in anxiety states is due to diversion of phenylalanine employed for adrenaline synthesis resulting in an autonomic inbalance.

Biological specificity and protein structure. DOROTHY WRINCH.[1]

Studies on the relation between biological function and form are already so far advanced that it is universally recognized that biological specificities belong to the angstrom world. There is no longer any doubt that the fundamental questions involved can be formulated—and subsequently studied and finally elucidated—only by recourse to the nature of the atomic patterns and electron density distributions. The studies of the crystal forms of about 170 different hemoglobins (Reichert and Brown, *The crystalline hemoglobins*, Washington, D. C., 1909) which have lain uninterpreted for nearly 40 years prove to yield material of the first importance for an understanding of biological specificities (Wrinch, *Am. Mineral.* in the press). The prevalence of twins and intergrowths among these protein crystals and their pseudosymmetries in many cases are found to relate them closely to a number of minerals for which the atomic patterns are already established. Attention is called to the strong case which can be made out for an isostructural relation between the oxyhemoglobin of *Necturus maculatus* and Staurolite ($H_2FeAl_4Si_2O_{12}$), between the oxyhemoglobin of *Cavite cutleri* and Tetrahedrite (Cu_3SbS_3). To interpret these striking facts, which stem from the forms of the crystals and the cube and double cube nature of the twins, trillings and other intergrowths, it is necessary to find a generalization of the structural essence of these minerals in terms applicable to proteins. Crystallographic studies have demonstrated how (e.g.) the carbon *atoms* in diamond may be replaced by *molecules*, in Sernarmontite or Arsenolite or the 29-hydrate of phosphotungstic acid (Wrinch, *Phil. Mag.*, **38**: 373, 1947: Wallerstein Communications in the press). By means of a basic principle of successive generalization, these molecules may, in turn, be replaced in appropriate circumstances by regular crystal-like arrays of molecules to give particles, crystals and intergrowths in general. It appears, therefore, that the major cubic theme in Staurolite and Tetrahedrite should be interpreted for the hemoglobins as cubic skeletons of interlocked α-levo amino acid backbones. The accompanying minor theme, which may or may not be cubic, is then seen to mean the R-substituents on the skeleton—the "fluff" or "spines" on the surfaces of protein molecules—together with the accompanying foreign molecules or ions in the crystal.

The postulate of a cubic arrangement of interlocked backbones in protein skeletons points the way to orderly arrangements of molecules into particles. Particles in the cubic and double cube systems, sometimes with disturbing non-cubic "fluff," containing 2, 3, 4, 5, 6, 7, 8, 12, 16, . . . molecules are suggested for consideration in the cases of the hemoglobins, insulin, horse serum albumin, ribonuclease, etc.

The picture suggested by the hemoglobin data thus includes (a) a general aspect embodying the protein essence in a cubic skeleton for the individual molecules, a number of molecules (possibly 12 with 48 residues apiece in many different hemoglobins) representing each individual particle and (b) a particular aspect embodying the protein specificity which resides in (b1) the

[1] Smith College.

pattern and associational complexes of the fluff or spines emerging from the skeletal surfaces and in (b2) the resulting characteristic differences in molecular patterns. In the forms of individual crystals and in the forms of their intergrowths in general lies a direct approach to the biological specificities of different hemoglobins *at the angstrom level.*

(This work is supported by the Office of Naval Research under contract N8onr-579.)

AUGUST 17

The incorporation of carbon dioxide into organic linkage by retina. R. K. CRANE, E. G. BALL, AND A. K. SOLOMON.

The incorporation of carbon dioxide into organic linkage first demonstrated in bacteria by Wood and Werkman (*Biochem. J.*, **32**: 1262, 1938) and in pigeon liver by Evans and Slotin (*J. Biol. Chem.*, **136**: 301, 1940) has since been shown to proceed by the beta carboxylation of pyruvic acid (Wood-Werkman Reaction). The incorporation first observed in the whole rat by Hastings et al. (*J. Biol. Chem.*, **140**: 171, 1941) has been assumed to occur by the same pathway. The present investigation confirms and extends the previous observations on avian tissues and provides a more direct demonstration of this reaction in the mammal.

Tissues were incubated with pyruvate at 37° C. in a closed vessel containing carbon dioxide and bicarbonate labelled with carbon fourteen. Excess pyruvate was isolated as the 2,4-dinitro phenylhydrazone. The hydrazone was recrystallized and its radioactivity determined. In a survey of various tissues under standardized conditions the following rates of CO_2 incorporation were found: pigeon liver, 37; duck retina, 17; ox retina, 11; rat liver, 4; rat kidney, 3; pigeon heart, 0.6; rat heart, 0.5; and pigeon breast muscle, 0.3. Retinas were used whole, all others were sliced. The extensive damage on slicing may account for the low rate in muscular tissues.

Since ox retina was the most active mammalian tissue, it was studied further. The rate of incorporation by this tissue was found essentially constant for at least four hours. Varying the CO_2-bicarbonate buffer system showed that optimum conditions exist when the bicarbonate ion does not exceed 20 millimols per liter. Increases above this decrease the rate whether the pH is or is not held constant by increasing the CO_2 tension. Within the range studied, pH (7.1–7.7) and CO_2 tension (5–20 per cent) appear to have little influence. Anaerobiosis reduced the incorporation rate by 70 per cent with no additional effect on the addition of 0.01 molar iodoacetate. The same concentration of iodoacetate added aerobically caused a 60 per cent reduction. Ammonium ion (0.01 molar) produced a 70 per cent reduction under aerobic conditions.

Ultrastructure of the nerve axon. E. DEROBERTIS (no abstract submitted).

Mechanisms of interaction of inhibitions with plasma cholinesterase. A. GOLDSTEIN (cf. Lalor Reports).

The synthesis of nucleoproteins in developing Arbacia studied with the aid of P^{32}. CLAUDE A. VILLEE, M. LOWENS, M. GORDON, E. LEONARD, A. RICH (cf. Lalor Reports).

GENERAL SCIENTIFIC MEETINGS

AUGUST 24

Enzyme localization in the giant nerve fiber of the squid. BENJAMIN LIBET (cf. Lalor Reports).

Choline esterase choline acetylase ratio in invertebrate tissues. HAROLD PERSKY (cf. Lalor Reports).

Non-integrative synapses. THEODORE H. BULLOCK.[1]

Synapses have been defined as valves which must change their conditions of openness from time to time in ordinary functions, or remain but partially open. That is they must integrate (make each presynaptic impulse count for more or less than one in eliciting an outgoing discharge Prosser). Until recently this concept could not be denied on grounds of known neuroneural junctions. There are now several synapses found among invertebrates which behave somewhat like the classical vertebrate neuromuscular junction: they apparently act normally as simple relays, passing every impulse that arrives in a 1:1 manner.

The giant synapse in the stellate ganglion of the squid may be such a case. Except in advanced stages of fatigue no summation, spatial or temporal, occurs, facilitation and after discharge are absent, though it is still possible that different paths as yet undetected physiologically may alter this. The junctions between central giants and motor giants in the ventral cord of the crayfish act in this simple relay fashion (Wiersma). There are evidently commissural synapses between the two lateral giants in the same animal which normally conduct in a 1:1 and, furthermore, an unpolarized manner. Finally, the septal junctions which recur segmentally in these same crayfish laterals and in the earthworm giant fibers act likewise until severely fatigued. These septa seem likely, on the basis of accumulating evidence (unpublished), to be real functional as well as anatomic barriers and therefore synapses on a less arbitrary definition.

What biologic meaning can such relay junctions have? Unless new afferents are formed must they be regarded as present only for the occasions when fatigue is advanced, or as trophic boundaries, embryonic rests or the meeting of incompatible protoplasms (these same species have demonstrated that neuronal fusion can occur in suitable situations)? Are they primitive or specialized junctions? Whatever their interpretation it seems necessary in the face of new evidence to broaden our conception of the synapse.

Phosphagen in annelids (Polychaeta). ERNEST BALDWIN AND WARREN H. YUDKIN (cf. Lalor Reports).

Crustacyanin, the blue carotenoid-protein of the lobster shell. GEORGE WALD, NEAL NATHANSON, WILLIAM P. JENCKS AND ELIZABETH TARR.[2]

It has been suggested that the striking change in color which the lobster shell undergoes on boiling is caused by the splitting of the red carotenoid astaxanthin from a blue complex with protein (Kuhn and Sörensen, *Ber. deutsch. chem. Gesel.*, 71: 1879, 1938). Kuhn and coworkers, however, were unable to extract such a complex from the shell, in which they believed it to be held by calcium deposits.

We have extracted a deep blue astaxanthin-protein from the lobster shell with dilute citric acid. This substance, called crustacyanin (i.e., shell blue), possesses a broad absorption band maximal at about 625 mμ. It is precipitated from solution by 40 per cent saturation with ammonium sulfate, and dissolves in distilled water. Its isoelectric point lies below pH 4.5. On denaturation by heating at 100° C. or treatment with mineral acids, alcohols or acetone in the warm, the blue solutions turn orange-red, the absorption maximum moving to about 460 mμ. The denatured solutions, on dilution with acetone or alcohol, yield all their color to neutral fat solvents in the form of astaxanthin.

If crustacyanin is warmed to 60° C. in m/10 veronal buffer, pH 7, it exhibits a type of reversible denaturation. The color goes from blue to purple, the absorption maximum from 625 mμ to about 530 mμ. On cooling, the color returns to blue, the absorption band to its previous position.

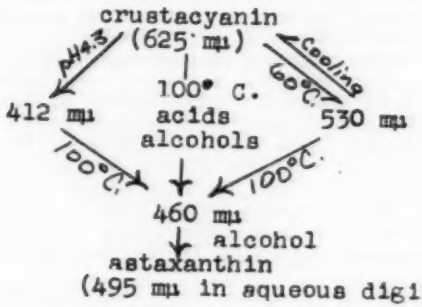
Crustacyanin preparations frequently display a narrow absorption peak at about 412 mμ.

[1] University of California, Los Angeles.

[2] This investigation was supported in part by the Medical Sciences Division of the Office of Naval Research.

This is very variable in height and in our best preparations is completely absent. Some very close relation exists between the 625 and the 412 mμ absorptions. When crustacyanin is brought to pH about 4.3 the 625 mμ band falls while the 412 mμ peak rises proportionately. The final product possesses the 412 mμ band alone. If this is heated it goes over to the orange-red color and 460 mμ absorption of heat-denatured crustacyanin.

These relations are summarized in the following diagram:



The activity and distribution of desoxyribonuclease and phosphatases in the early development of Arbacia punctulata.[1] DANIEL MAZIA, GERTRUDE BLUMENTHAL, AND ELEANORE BENSON.

The original aim of this investigation was to find enzymes that were associated with the nucleus and to follow their behavior through nuclear division. Desoxyribonuclease (DNase) seemed a logical choice because its substrate is restricted to the nucleus. There has also been evidence of nuclear localization of phosphatase (Pase). DNase was determined by the method of Barton and Mazia, involving separation of the high polymer DNA by precipitation with protamine. In the Pase measurements, glycerophosphate was used as substrate and inorganic P was determined.

Nucleated and enucleated fragments of unfertilized eggs were obtained by the Harvey method. There was no clear indication of sharp nuclear localization of acid Pase (pH 5) or alkaline Pase (pH 9). Desoxyribonuclease was not restricted to the nucleated fragments. These generally had a higher activity per fragment, but not per unit volume. The sum of the activities of the fragments was 30–40 per cent higher than that of the whole eggs. It is indicated, therefore, that most of the DNase activity of the unfertilized egg is in the cytoplasm, though DNA has not been found there in measurable amounts.

The DNase activity was followed through developmental stages to the 40 hour pluteus. Total activity and the activity of the "soluble" fraction (not sedimentable at 20,000 g.) were measured. Total activity did not increase significantly during development. The non-sedimentable fraction declined steadily, reaching at 40 hours a value 10–20 per cent of the total activity. The enzyme seems to be fixed on structural components of the cell, possibly nuclei, as development proceeds.

In the unfertilized egg the acid Pase predominates. The activity is 3–5 times that of alkaline Pase. During development, acid Pase remains constant. Alkaline Pase remains constant until just before gastrulation, then rises steadily to a value 10 or more times that of the unfertilized egg.

These findings make no contribution to the original question of the behavior of nuclear enzymes in cell division but do show the existence of two different patterns of development of enzyme systems in morphogenesis. One is the formation of new enzyme (alkaline Pase) in the course of development. The other is the storage of adequate amounts of enzymes (DNase)

[1] Work supported by grants from NRC Committee on Growth, acting for the American Cancer Society, and from University of Missouri Research Council.

during oogenesis and the subsequent structural differentiation of the enzyme as morphogenesis proceeds.

The effects of nitrogen mustards on cleavage and development of Arbacia eggs. JOHN O. HUTCHENS AND BETTY PODOLSKY.

Effects of tris(β-chloroethyl) amine; bis(β-chloroethyl), methyl amine; bis(β-chloroethyl), isopropyl amine; n-butyl, bis(β-chloroethyl) amine; benzyl, bis(β-chloroethyl) amine; bis-(β-chloroethyl), β-methoxyethyl amine; and bis(β-chloroethyl) furfuryl amine on early cleavage and developmental stages attained at 12, 24, and 48 hours were studied using fertilized eggs of *Arbacia punctulata*. Amine hydrochlorides dissolved in 0.52 M NaCl were added to suspensions of eggs in sea water 20 minutes after fertilization. One hour after exposure the eggs were washed twice in sea water. Temperature was 22° C.

Nitrogen mustards in non-cytolyzing concentrations slow, rather than completely block, division. At 3 hours only about 50 per cent reduction in number of cleavages can be effected without cytolysis. Concentrations of various compounds reducing cleavage to 75 per cent of the control value at 3 hours were therefore compared. Tris(β-chloroethyl) amine is most effective, a dose of 2.8×10^{-6} M being required. The various bis(β-chloroethyl) amines are all about one-tenth as effective, doses of $2\text{–}3 \times 10^{-4}$ M being required.

Cleavage in the presence of nitrogen mustards was frequently irregular, some blastomeres dividing more slowly than others. If cytolysis did not occur, apparently normal ciliated blastulae eventually developed. Gastrulation was blocked, however, even at concentrations which only slightly inhibited early cleavage. 10^{-5} M tris(β-chloroethyl) amine or 10^{-4} M solutions of the other six compounds prevented normal pluteus development at 24 hours. That micromere formation and setting aside of proper proportions of animal and vegetal cells is not involved is indicated by the fact that ciliated blastulae (ca. 8 hrs. development) exposed to these same concentrations were inhibited to about the same extent at 24 hours.

Respiration of the fertilized egg is not inhibited significantly by these concentrations of nitrogen mustards. Respiration of treated embryos, in fact, increased with time, corresponding to the developmental stages attained.

The effects of pressure on the insemination reactions of Arbacia eggs. DOUGLAS MARSLAND.[1]

A syringe placed inside the microscope-pressure chamber permits sperm to be ejected upon the eggs exactly 10 seconds before pressures up to 15,000 lbs./in.^{2} are applied. At higher pressures (6000 lbs. and above) the egg gives no visible reaction to the sperm. Numerous active sperm may come into contact directly with the egg surface, but the fertilization membrane does not lift and the hyaloplasma layer does not appear so long as the pressure is maintained. But as soon as the pressure is released, the fertilization membranes and the hyaloplasma layers begin to appear; and subsequently the eggs develop normally, at least to the free-swimming stage, provided the period of high pressure does not exceed about 15 minutes. However, the lifting of the fertilization membranes in the pressure treated eggs is slower and less complete than in untreated eggs; and for pressures exceeding 8000 lbs., no fertilization membranes can be distinguished on most of the eggs, although the hyaloplasma layer becomes distinctly visible after the pressure is released, and the cleavages go ahead on schedule. Also pressures above 6000 lbs./in.^{2} must reversibly block the penetration of the sperm into the egg, since control eggs fertilized at the instant when the pressure is released undergo the first cleavages in exact synchrony with eggs which had been fertilized earlier and then immediately exposed to a 15 minute period of compression.

Below 6000 lbs./in.^{2} the pressure inhibition of the insemination reactions is less complete. At 4000 lbs. the sperm can penetrate the egg surface during the compression period and the hyaloplasma layer can form, although the lifting of the fertilization membrane is suppressed in almost all the eggs until the pressure is released. And while a pressure of 2000 lbs. is main-

[1] Professor of Biology, Washington Square College of Arts and Science, New York University.

tained, the only observable effect is a delay in the penetration of the sperm, the formation of the hyaline layer, and the lifting of the fertilization membrane.

Lactones as mitotic poisons, tested on sea urchin eggs. IVOR CORNMAN.

The lactone ring is prominent in many physiologically active compounds produced by plants, unsaturated lactones being especially active. Experiments with the eggs of *Lytechinus variegatus*, beginning exposure 10 minutes after fertilization, show that effectiveness in blocking cleavage increases 1000-fold with shifts in the position and number of the double bonds. Alpha angelica lactone (3-pentene-1,4-olide) slows the second cleavage 4 per cent at 0.11 mMolar, and blocks eggs in the 2-cell stage at 1.13 mMolar. Moving the double bond to carbons 2 and 3 (β angelica) decreases the activity by a factor of 10. However, adding a double bond (2,4-pentadiene-1,4-olide: protoanemonin) gives a compound which blocks all cleavage at 0.01 mMolar. Coupling two of these molecules at carbons 4 and 5, thereby destroying the second unsaturated linkage (anemonin) reduces the activity: blocking at 0.52 mMolar. A comparable decline in potency comes with moving the 4,5 double bond farther out a side chain (γ-propenyl-γ-crotonolactone) or hydrolysing it (2-pentene-4-hydroxy-1,4-olide).

At these concentrations which block cleavage, there is first a development of the achromatic figure. It is then inactivated, as evidenced by the incomplete separation of anaphase chromosomes in α-angelica lactone. Flakes of hyaline material in eggs treated with anemonin and protoanemonin suggest dispersal of the achromatic figure without its complete resorption. Studies have already been made of the effects of lactones on plant mitoses (Erickson and Rosen, *Science*, in press).

Penetration and effects of low temperature and cyanide on penetration of radioactive potassium into the eggs of Strongylocentrotus purpuratus and Arbacia punctulata. E. L. CHAMBERS, W. WHITE, NYLAN JEUNG AND S. C. BROOKS.[1]

Radioactive potassium was added to 0.2 per cent suspension of (1) unfertilized, and (2) fertilized eggs of *S. purpuratus* at 15° C., giving a [K^{42}] of .015 μc/ml. Samples were removed at intervals for determinations of radioactivity, and for chemical analyses (cf. method, abstract presented by title, Penetration of Radioactive Phosphate, etc.).

In the unfertilized egg the exchange rate is gradual. The total quantity of K^{42} entering reaches a maximum in 15 hrs. At this time the specific activity of the eggs: $\frac{[K^{42}\text{ inside}]}{[K^{39}\text{ inside}]}$ equals 20 per cent that of the suspension fluid: $\frac{[K^{42}\text{ outside}]}{[K^{39}\text{ outside}]}$.

In the fertilized egg the exchange rate increases immediately, the maximum being reached in 30 minutes. At the time of first cleavage (110 minutes) the specific activity of the eggs had reached 50 per cent that of the suspension fluid. During this period no increase in content of K occurred. A maximum is reached in 15 hrs. when the specific activity approaches about 85–90 per cent that of the suspension fluid. During the first two hours of development (one cell stage) the total exchange rate of K in the fertilized egg was found to be 7–13 times that of the unfertilized.

The tentative assumption is that the total quantity of readily exchangeable K approximates 20 per cent in the unfertilized egg and 85 per cent in the fertilized. By making the calculation on this assumption the rate of approach to equilibrium of the freely diffusible K is only 2–3 times more rapid in the fertilized egg. A similar relation was found in the eggs of Arbacia.

Exposure of the fertilized *purpuratus* eggs for a short period to low temperature, and to cyanide, pronouncedly decreased the rate of exchange without altering the K content. The Q_{10} (8.5° C.–18.5° C.) for cleavage time was 2.4, and for the exchange rate of potassium 2.0. NaCN was adjusted, immediately before use, to pH 8.4 and then added to hermetically closed flasks containing egg suspension. Concentrations ranging between 1×10^{-4} to 1×10^{-3} M caused a 2–3 fold decrease in rate of exchange with complete inhibition of cleavage. The eggs showed 100 per cent recovery when returned to sea water.

[1] Department of Zoology, University of California, and Eli Lilly Research Laboratories, M. B. L., Woods Hole, Mass. Under grant from N. C. I., U. S. P. H. S.

In conclusion, before evaluation can be made of permeability differences between fertilized and unfertilized eggs, it is necessary to take into account the findings that the two types of eggs differ in their possession of relatively non-exchangeable potassium.

Cartesian diver technique: a simplified mixing method in a new type of cartesian diver vessel. C. LLOYD CLAFF AND T. N. TAHMISIAN.

Problems in manipulation of Cartesian divers where solutions must be mixed after an initial trend has been established are simplified. A new type of Cartesian diver vessel is introduced which has an "hour glass" shape. It can be fashioned without difficulty in the Diver Jig described by one of us (C. L. C.) in *Science* (Vol. 107, February, 1948). The lower chamber is used as an air expansion chamber, and the alkali drop is placed therein when necessary. The upper chamber is used for one of the reaction components and the lower end of the neck is used for the second reaction component, and when necessary a third component. The oil seal is placed in the usual position. The oil is colored with Sudan III. The KOH is colored with phenolphthalein. The dyes simplify observation: Sudan III in the oil makes it possible to see admixture of flotation medium should such an accident occur.

The mixing of the reaction solutions is accomplished by over-pressure applied from a sphygmomanometer bulb and controlled by the sphygmomanometer bulb valve. The overpressure is applied to the manifold system only. The manometer proper is closed off by a three way stopcock.

Type experiments involving evolution of CO_2 from quantitative mixture of $NaHCO_3$ and H_2SO_4; normal respiration, toxicity of *Chaos chaos* to uranyl nitrate; subsequent recovery by addition of phosphate-citrate to uranyl nitrate treated *Chaos chaos;* enzymatic catalysis of CO_2 from pyruvate; fertilization of Arbacia eggs; as well as respiration of *Paramecium calkinsi,* before and after mixing of Types I and II, were studied and graphs of results presented.

Fixation and staining of plant nuclei in lacto-sudan black b. ISADORE COHEN.

Sudan Black B (SBB) is soluble in 85 per cent lactic acid, giving a deep reddish-brown solution (LSBB). Chromatin is stained brown by this mixture. SBB is a hydrophobic dye and precipitates out in the presence of excess water. Fixation and staining of freehand sections of onion bulb in LSBB showed that the resting nuclei in various tissues of the plant differed in their fixation image. Nuclei with heavy coarse reticula represented one extreme and were related by a series of gradations to epidermal cell nuclei with remarkably sharp chromonematic structure. In the resting nuclei of root tips of the lima bean, the chromocenters stain red while the fine chromatin threads connected to them stain light brown.

In onion root tip smash preparations made with LSBB, late prophase, meta- and anaphase stages were as a rule so poorly preserved that they were hardly recognizable as such. The spindle was not fixed and the chromosomes appeared to be despiralized and elongated. Despiralization seems to occur also in resting and early prophase nuclei. It is suggested that this despiralization might explain the accentuated chromonematic fixation image given by 85 per cent lactic acid.

Smash preparations of onion root tips, prefixed for one-half hour in Carnoy's fluid, were made using the mixture of 2 parts of LSBB and one part of N-butyl alcohol slightly undersaturated with about 8 per cent water. Large numbers of nuclei in various stages of the mitotic cycle are thus released into the medium. Frequently, in moderately despiralized chromosomes there are seen exceptionally clear cut chromonematic structures. Nucleoli and cytoplasm do not stain. Onion epidermal cell nuclei fix and stain well in this LSBB and N-butyl alcohol mixture. When viewed with a blue daylight filter in combination with a Wratten X-1 number 11 green filter the chromosomes and nuclei appear black against a green background.

Urea reabsorption in the smooth dogfish kidney. RUDOLF T. KEMPTON.

Urea reabsorption by the kidney tubules of the smooth dogfish, *Mustelus canis,* has been studied by the use of inulin as a measure of glomerular filtration. In most of the experiments the reabsorption of water, urea and glucose was determined simultaneously. A total of 58 collec-

tion periods in 17 different animals has dealt with urea at the normal blood levels; in 8 other collection periods with 4 animals the urea blood level was raised by the administration of very heavy doses of this substance.

There has been a surprisingly wide range of urea levels in the plasma of freshly caught animals, varying from 745 mgm. per cent to 2100 mgm. per cent urea nitrogen (including any ammonia nitrogen) or in other words urea levels from approximately 1.6 to 4.5 per cent. When the average rate of reabsorption of urea per 100 cc. of filtrate is plotted against the plasma urea level, there results a straight line which is parallel to the filtration rate. On the average, therefore, there is essentially a constant amount which is not reabsorbed from the filtrate. This does not result in a constancy of urea concentration in the urine because of the great variations in the concomitant reabsorption of water. It seems to be clear that the rate of urea reabsorption is therefore determined in some manner either by the amount of non-reabsorbed urea left in the tubule or by the plasma level. There are at present no data which give a clear indication of the proper choice of alternates, although some of the facts tend to point toward the latter as the controlling influence.

In animals in which the urea level has been raised by heavy injections of urea, there is a decrease in the reabsorption of urea in relation to the plasma level, in spite of the fact that there is no similar decrease in water and glucose reabsorption. However, in seven of the eight collection periods, if reabsorption per 100 cc. of filtrate is plotted against the plasma level prevailing before the urea injections were made, rather than afterward, the points fall in line with the other experiments. This would indicate, if further experiments now in progress substantiate this relation, that the rate of urea reabsorption has been "set" at the previous normal urea level; and that the rate did not become markedly modified during the period in which the urea level was elevated.

The influence of theophylline on the absorption of Mg-salts from the gastro-intestinal canal. A. FROELICH.

Magnesium sulphate ($MgSO_4 + 7H_2O$) is an excellent laxative. When orally administered it is not absorbed; no cases of Mg-poisoning have ever been reported.

After injection (s.c., i.m., or i.v.) a condition of "Mg-Narcosis" develops (Meltzer and Auer) easily counteracted by intravenous injection of soluble Ca-salts. When $MgSO_4 + 7H_2O$ was brought into the stomach of normal frogs (*R. pipiens*) in 25 per cent solution (ca. 0.5 cc. per 20 g. frog) nothing happened. But in frogs who had one hour before received a single injection of Theophylline-sodium-acetate (0.15 mg. pro gm.) into a lymph sac, the same amount of Mg-sulphate produced within 10–20 min. the condition of "Mg-Narcosis." Frequently the frog died within the following 24 hours. The absorption took place chiefly from the stomach and to a minor part from the rectal part of the intestine, as could be shown in frogs whose pylorus had been previously ligatured.

The Mg-chloride ($MgCl_2 + 6H_2O$) when given by mouth in a 20.5 per cent solution in a quantity similar to that of $MgSO_4$ is already absorbed from the gastro-intestinal canal, without previous injection of theophylline. This is in accordance with the findings in large mammals (sheep, goat): when big doses of $MgCl_2 + 6H_2O$ were introduced into the stomach of normal animals, this led to "Mg-narcosis."

However, experiments performed on rats during the winter of 1947–48 at the May Institute for Medical Research in Cincinnatti, O. (Director, Dr. A. Mirsky), gave results which differed in some respects from those obtained on frogs at the Marine Biological Laboratory, Woods Hole, Mass., during July and August 1948.

Both $MgSO_4 + 7H_2O$ and $MgCl_2 + 6H_2O$ were without any visible effect after introduction into the stomach of normal rats weighing from 100 to 250 gms [$MgSO_4$ in doses of 2 or 3 mg. per 50 g. rat, MgCl in isoionic quantities (10 per cent)]. After previous s.c. injection of theophylline-sodium-acetate (0.15 mg pro gm.), the sulphate when given orally produced pronounced "Mg-Narcosis" leading in some cases to the animal's death. But the introduction of $MgCl_2$ in isoionic quantities was found to be without this effect as well with or without previous treatment with theophylline. An explanation for this cannot be offered so far. For the occurrence of "Mg-Narcosis" after introduction into the stomach of both frogs and rats under the action of theophylline, increased permeability leading to absorption undoubtedly is responsible (A. Froelich and E. Zak).

The relative rate of penetration of the lower fatty acids into erythrocytes of the smooth dogfish. JAMES W. GREEN.[1]

The penetration of a weak acid into erythrocytes is accompanied by a dissociation of the oxyhemoglobin to a new equilibrium. This dissociation of the oxyhemoglobin may be detected spectrophotometrically. Using a modification of the Hartridge-Roughton rapid mixing technique applicable to a spectrophotometer, a relative measure was made of the rate at which the saturated, monocarboxylic acids from formic through caprylic entered the red cells of the smooth dogfish. The method measures a chemical and not a volume change. Dilute, unbuffered red cell suspensions in sea water were mixed with dilute acid solutions also in sea water. The pH of the final mixtures was approximately 4.5 to 5.0.

The relative order of increasing rate of penetration of these acids was found to be: formic, acetic, propionic, caprylic, heptylic, caproic, butyric and valeric. This was the same order as was found for beef red cells in earlier work. The time in seconds to 50 per cent penetration of the dogfish cells was found to be: formic, 5.72; acetic, 0.89; propionic, 0.21; butyric, 0.019; valeric, 0.019; caproic, 0.054; heptylic, 0.12; caprylic, 1.44.

The times to 50 per cent penetration for acetic, propionic, butyric, valeric and caproic acids were markedly faster than the corresponding times in beef red cells. Since these acids, according to the Overton theory of lipid solubility, are thought to penetrate cells through the surface lipids it is suggested that the increase in penetration rate (over that found in beef cells for these acids) may be correlated with the greater lipid content of the fish cell ghosts as shown by the analyses of Dziemian. The penetration rate of the heptylic and caprylic acids is slower than expected on the basis of their lipid solubility. It is suggested that the molecular volume of these two acids offers a barrier to their entrance into these cells.

The effect of bacterial toxins on the permeability of dogfish erythrocytes.[2] F. R. HUNTER, JANE A. BULLOCK AND JUNE RAWLEY.[3]

As one aspect of the general problem of the action of bacterial toxins on the functioning of cells, the effect of a number of toxins on the permeability of dogfish (*Mustelus canis*) erythrocytes was studied. Using a photoelectric technique to measure hemolysis times, little or no change in permeability to ethylene glycol was noted when dogfish erythrocytes were exposed to the following toxins for periods of time up to 24 hours: *Clostridium septicum, Clostridium tetani, Staphylococcus aureus* and *Corynebacterium diphtheriae.* The toxin of *Clostridium perfringens* caused a marked decrease in hemolysis time in ethylene glycol almost immediately (less than 10 min.). The toxins of *Bacillus cereus* and *Streptococcus pyogenes* caused a decrease in hemolysis time after several hours (6–10 hours) exposure. It is hoped that a further analysis of these data and a comparison with comparable data obtained from studies on chicken erythrocytes, *Asterias* and *Arbacia* eggs may give some information as to the action of toxins at the cellular level.

New experiments and observations on sexual instability in Crepidula plana. HARLEY N. GOULD AND SIDNEY C. HSIAO.

This report involves further attempts to clear up the mechanism of self-regulation of sex in communities of *Crepidula plana.* In isolation-experiments, some of the small, sexless individuals confined in separate glass tubes in the harbor water at Woods Hole have developed a temporarily functional male phase, as found by Coe, while control cultures in running sea water in the laboratory have disclosed at most only a partial male development. The difference does not appear to be of nutritional origin, as suggested by Coe, because (a) cultures in running sea water show equal or greater rapidity of growth compared with identical cultures in the

[1] Physiological Laboratory.

[2] This work was supported by grants from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service and the Faculty Research Fund, The University of Oklahoma.

[3] Marine Biological Laboratory, Woods Hole, Mass., and the Department of Zoological Sciences, The University of Oklahoma, Norman.

harbor, and (b) young sexless individuals confined in tubes with females in running sea water in the laboratory develop rapidly into adult males.

Congenital differences in growth rate and sexual development appear in every culture of equal-sized young. This forms the basis for the presence of both sexes in the community, which is later assured by the effect of the more rapidly growing individuals on the smaller ones.

Individuals of comparatively rapid growth under isolation, apparently tending toward direct female development, can be induced to develop into adult males by associating them with immature or mature females.

Males which after long isolation have lost external male characters have regained full male development after eleven days association with females, and sections of the visceral sac demonstrate evidence of internal sexual atrophy followed by regeneration.

In one-fourth of the cases, males from which the phallus has been removed by operation, when kept in association with females, regenerate the organ completely, and in another one-fourth, partially, after nine days. Males isolated after the operation show much less regenerative ability.

These experiments are continuing, and investigations of starvation and of hormone effects are under way.

The doubtful character of "break" excitation in skeletal muscle. PAUL G. LEFEVRE.

It was previously reported that nerve-free regions of the frog sartorius and pharyngeal retractor of *Thyone* failed to respond at "break" of a constant current. This was in accordance with absence of accommodation in these tissues, in light of excitation theory. Since this report, several papers from Dittler's laboratory at Marburg have dealt with response of curarized muscles to interruption of a steady current.

For this reason, the matter was briefly reinvestigated on gastrocnemii from frogs, in some of which a section of the sciatic nerve in the lower thigh had been removed 2–4 weeks previously. These muscles were stimulated through non-polarizable calomel half-cells by means of a specialized generator of pulses of about 2 seconds' duration; the automatic keying, tested oscillographically, was free from disturbing transients. The isometric response was observed oscillographically by amplification of current from a Statham strain gauge attached to the tendon of the slightly stretched muscle.

Muscles containing intramuscular nervous elements responded in the classical manner, with a sharp development of tension at "make" and "break" of the current and some degree of maintained tension during its passage, depending on current intensity. "Break" response typically occurred with current intensities over 3–5 times threshold for "make" response. In denervated muscles, no "break" response was seen even at intensities up to 15 times "make" threshold. The "break" response was not, however, simply attributable to involvement of intramuscular nervous elements, as it persisted in muscles exposed to *d*-tubocurarine in doses blocking neuromuscular transmission.

Since the capacity for "break" response appears to be neither intrinsic in denervated muscle nor dependent on neuromuscular transmission, it is suggested that it is a property of a specialized region of the muscle at the neuromyal junction or of a special structure there interposed.

Comparison of frog nerve and squid axon with respect to the measurement of accommodation. PAUL G. LEFEVRE.

The previous report of the occurrence of "break" response in the absence of accommodation, in decalcified frog nerves, was based on observation of the muscular response as index of nerve activity. Some difficulties of interpretation were avoided by use of a single-unit system, the squid giant axon, in an effort to check the phenomenon; but it proved impossible to alter the natural rate of accommodation in this cell by decalcification. The unaltered accommodation rate in the face of pronounced autorhythmic activity in the decalcified state invalidates the simple interpretations of this rhythmicity suggested by Solandt and Katz.

The pattern of response of the giant axon to alternating currents failed to conform with excitation theory. Curves relating liminal intensity with the logarithm of the frequency were symmetrical about the optimal frequency, in accordance with Coppée's observations on other

tissues; but there was consistent deviation from the predicted linearity in the relation between the squares of the liminal intensity and of the periods above the optimum.

The accommodation rate was extremely rapid in the giant axon; measured by stimulation with alternating currents, Hill's λ was not definitely measurable due to the deviation from theory, but was certainly less than 7 msec.; measured by stimulation with exponentially rising currents, $\lambda = 0.9 - 1.5$ msec., or only 2–4 times the factor for the excitatory process; whereas in frog nerve the two factors are of entirely different orders of magnitude.

The disagreement between the rates of accommodation measured on the giant axon in these two ways (which yield identical results in frog nerve), and the non-conformity of the squid axon with theories fitting the behaviour of the frog nerve are probably associated with observations on giant axons by Arvanitaki, Cole, and others, indicating that the response of the local excitatory state to subthreshold electrical disturbances is considerably more complex than as postulated in the excitation theories of Rashevsky, Hill, and Monnier.

August 25

Do genes exist? P. W. Whiting.

Johannsen named the gene and supposed it to be a "unit factor" or "element" "demonstrated by modern mendelian researches" and he suggested that it might be a side-chain of a large protein molecule. Recent speculation follows the same course, the gene being thus a corpuscular unit tracing back to the micella, the pangene, etc. It is here suggested that the word genes be used for terms in genetic formulae expressing mendelizing differences. It follows from this, somewhat in agreement with Goldschmidt's dissenting views, that the physical basis for genes is a considerable variety of chromosomal conditions. The germ plasma is genic material but it does not consist of genes. It produces genes by such structural reorganizations as may subsequently mendelize with the original condition. Usually this reorganization is very localized, giving rise to the linear order of the genes, but it may involve many regions provided these act as a unit of segregation in meiosis of the heterozygote. There is, however, no basis for assuming unified segregation in the homozygote. For example, the sex-differentiating gene pair of Drosophila mendelizes in the male, the heterozygote Mm (YX), but there is crossing-over between homologous parts of paternal m and maternal m in the female, mm (XX). The physical basis for the dominant gene M is the absence of one of the X chromosomes. Similar reasoning may be applied to gene pairs whose physical basis is known to be located in inversions, deletions, repeats, etc. Furthermore it seems preferable to extrapolate the nature of mendelizing pairs or series which are not microscopically demonstrable from those that are rather than to postulate the existence of a genic corpuscle by analogy with the subcellular units of past philosophies.

Dominant lethals induced by x-rays in sperm of the chalcidoid wasp Nasonia brevicornis Ashmead [= *Mormoniella vitripennis (Walker) fide Muesebeck in lit.*]. D. T. Ray.

In preliminary tests, 1287 ♀♀ and 249 ♂♂ (84.4% ♀♀) were bred from 8 mated mothers, 2097 ♂♂ from 11 unmated. Later 2117 ♂♂ were bred in 41 egg-laying days (51.6 per day) from 13 young unmated mothers and 1042 ♂♂ in 32 days (32.6 per day) from 4 ♀♀ exposed to males. In the x-ray experiment males were treated as freshly eclosed adults and were then paired with untreated females. The females were set with several blow-fly pupae and transferred every two or three days for three or four transfers. 119 egg-laying days of the controls yielded ♀♀ 4213 (35.4 per day), ♂♂ 911 (7.7 per day) (82.2% ♀♀). Data for the treated arranged as r units/egg-laying days/daughters/sons were 500 r/40/1013/270; 1000 r/49/1190/319; 1500 r/45/673/314; 2000 r/128/1326/903; 2500 r/16/95/133; 3000 r/49/341/404; 3500 r/24/84/245; 4000 r/90/200/700; 5000 r/60/50/540; 6000 r/128/24/1142; 7000 r/53/4/365; 8000 r/52/1/386; 9000 r/42/0/434; 10,000 r/34/0/342. Curve for ♀♀/total progeny is much steeper than that for Habrobracon (Heidenthal, *Genetics*, **30**: 197–205) but not as steep as that for Melittobia (Kerschner, *Anat. Rec.*, **96**: 556) because sex ratio of the Nasonia controls is intermediate. A close fit to the Habrobracon curve is obtained by multiplying by 70/82, the percentage of Habrobracon

females in controls divided by that of Nasonia. Sons, averaging 7.97 per day, are not directly affected by increasing dose, but with higher treatments of their "stepfathers" they show a slight increase in numbers and a considerable increase in size due to fewer sisters competing for food. Excluded from the above data are males produced after presumed exhaustion of the mothers' sperm supply. These totalled 2681 bred in 72 days, the average 37.2 contrasting with the low numbers appearing in earlier vials. Also excluded are 5 vials of diapause larvae produced by 2 mothers preceding and by 3 mothers following production of progeny with normal life span.

The use of diethylstilbesterol in the production of eye mutations in drosophila melanogaster. BURTON L. STEKLER.

The use of nitrogen mustard in the production of genetic mutations in *Drosophila melanogaster* is well known. However, there is no specificity in its action and like X-ray, produces the random type of mutation. In work along similar lines of investigation, it was found that diethylstilbesterol produced striking changes in the architecture of the Drosophila eye, and that these changes could be reproduced in succeeding generations.

Due to the insolubility of diethylstilbesterol in water, the ordinary corn meal-molasses medium was not used. Instead, a synthetic medium containing sucrose, inorganic salts and agar was substituted. The drug, in varying concentrations (1 mg.%–20 mg.%), was then added, and the food allowed to harden. Three or four drops of a water-yeast mixture were added to this.

Three different wild type stocks of Drosophila were used for the experiment. Two males and two females of each stock were allowed to mate on the drug-containing medium. Eggs were laid thereon and the larvae were forced to eat the drug-containing medium. The temperature was kept at 24° C. after the parents were put in with the experimental medium.

The F–1 generation was studied for mutations and it was found that the drug-treated Woodbury and Florida wild type flies exhibited a fairly regular eye mutation. The eye characters resembled lobed eye, but as yet this fact has not been proven. The percentage of mutants in the two wild type stocks varied between 0.4–0.7%. The Oregon wild type stock did not produce any mutations at 24° C. but did so at 29° C. Control groups of flies were run under the same conditions and the mutants were not observed to appear in 3500 individuals. These results substantiate previous data which were collected in 1946, in which the same experiment was tried. Mutants produced at that time have been carried on normal food since the F–1 generation and have so existed for 56 generations.

There are indications pointing to the Stilbene nucleus as being the important factor in this chemically produced mutation. This is shown as a result of adding stilbamadine, which is closely allied in chemical structure to diethylstilbesterol, to the synthetic medium, the same eye changes are produced, only at a greatly reduced frequency.

Much remains to be done in proving whether or not this drug is specifically directed and investigations will be continued toward this end.

Predictable mutations in bacteria. E. RUTH WITKUS.[1]

A colorless mutation has been produced in *Sarcina lutea*. The colorless mutation was obtained by mixing several different organisms in a liquid medium and growing the organisms together for twenty-four hours at 37° C. The following four species of bacteria were grown together in nutrient broth for twenty-four hours: *Bacillus subtilis, Bacillus megatherium, Proteus vulgaris* and *Sarcina lutea*.

After twenty-four hours the organisms were reisolated by the dilution method and five different strains instead of four were recovered. In addition to the four original species, a new non-pigmented form was always obtained. The new type is similar to *Sarcina lutea* in all morphological features except in its color. The yellow and white colonies appeared in a ratio of three to one.

If *Proteus vulgaris* was omitted from the mixture, a white mutant was also produced. If either *Bacillus megatherium* or *Bacillus subtilis* was omitted, no white mutant was produced. No white mutant was obtained when mixtures of only two organisms were used.

A mixture of four entirely different organisms was used—*Bacillus cereus, Corynebacterium xerose, Serratia marcescens* and *Sarcina lutea*. No white mutant was obtained in this instance.

[1] Biological Laboratory, Fordham University.

White mutants were also obtained by growing *Sarcina lutea* in a liquid medium containing a sodium salt of ribose nucleic acid.

Method of origin of androgenetic males in Habrobracon. ANNA R. WHITING.

1.57 per cent of eggs x-rayed in first meiotic metaphase and fertilized by untreated sperm develop into normal fertile haploid androgenetic males. Although lethal dose for the egg nucleus in this stage is about 2,400 r, androgenetic males occur after treatments as high as 54,000 r. Of 291 eggs in which origin of cleavage nuclei could be determined, 3 or 1.03 per cent showed cleavage of sperm nuclei only. In 3 others, incipient androgenesis was strongly suggested. The maximum number of 6 or 2.04 per cent compared with 1.57 per cent of adults emerging shows that androgenetic larvae do not differ in viability from larvae developing in untreated eggs. Chromatin bridges in meiotic division II which occur following treatment in metaphase I allow androgenetic development when they retard the egg nucleus to such a degree that it does not reach the sperm nucleus before cleavage begins. The rarity of such bridges in eggs following treatment in prophase I will explain why no androgenetic males developing from these eggs have been found.

X-Radiation effects on the restitution of dissociated Microciona. C. K. LIU.[1]

Dosages of 10,000 r, 25,000 r, 36,000 r and to some extent 50,000 r, given to *Microciona* immediately before squeezing it through bolting silk with meshes of 40 μ, produced little effect on the process of restitution.

Dosages of 100,000 r to 300,000 r were excessive. With 300,000 r, aggregation still occurred but stopped at the spheroid stage and the aggregates died after 4 days. With 100,000 r and 200,000 r aggregation proceeded to an ill-defined reticulum stage. The cell mass then retracted from the surrounding hyaline layer and collected into groups of rounded-up cells. Some of the masses survived for 3 weeks, but no differentiation took place.

The dosage of 72,000 r produced significant results and was studied in more detail. Irradiation was done on: (1) sponges just before being dissociated; (2) suspension immediately after dissociation; (3) restituted sponges 10 days after dissociation when large numbers of flagellated chambers were formed.

In (1) and (2) aggregation occurred normally but, after the reticulum stage, retracted instead of going into the stage of spreading. The retracted condition persisted until the 11th day when the spreading out began to occur. Flagellated chambers appeared on the 14th day instead of on the 4th day as in the control.

As an immediate result of (3) many flagellated chambers actually disappeared, presumably due to their breaking down into separate choanocytes. The remaining chambers collapsed. There was a decided loss of definition of all cells. Retraction of the colony soon followed and showed 50 per cent reduction in size at the end of 24 hours, leaving spicules behind. The colony retrogressed into a mass of cells with no structural differentiation. The nucleolated archeocytes became filled with coarse orange granules, presumably ingested choanocytes. The colony remained retracted for 10 days when it again spread out. Flagellated chambers began to form on the 14th day. Their subsequent development showed no superiority in number or size over (1) and (2).

By comparing (1), (2) and (3), one can conclude that the regeneration of the flagellated chambers in the irradiated material is independent of their state of development at the time of irradiation. The fact that the flagellated chambers formed at the same interval of time after irradiation and increased at the same tempo suggests that the choanocytes are formed *de novo* from the archeocytes.

Combined effect of ultraviolet light and heat upon first cleavage of Arbacia eggs. LOIS M. HUTCHINGS.

Ten minutes after insemination, Arbacia eggs were exposed for 1, 2, 3, 4, or 5 minutes to a temperature of 36° $\pm$ 0.05° C. or were given 40, 80, 120, 160, or 200 seconds of 2537 Å ultraviolet

[1] Laboratory of Experimental Cell Research, M. B. L., Woods Hole.

light. Other eggs were given serial dosages of heat preceded or followed by 10 seconds exposure to ultraviolet light, and still others received serial dosages of ultraviolet light preceded or followed by 120 seconds heat at 36° C. Thus, in addition to controls there were six phases to a complete experiment. All dishes were kept on a water table whose temperature variation during the experiment was $\pm$ 0.15° C. The time at which 50 per cent underwent first cleavage was determined through several counts of 100 eggs per count.

Thermal treatment applied to Arbacia eggs in serial dosages shows a continuous progression from mild to severe inhibition, which increases markedly after 3½ minutes of heating. Serial dosages of 2537 Å ultraviolet light produce an intense initial inhibition of cleavage followed by a mild secondary inhibition which increases slowly in proportion to dosage. Treatments involving both irradiation and heat inhibit cleavage more than either agent alone. Curves of treatments involving irradiation and serial dosages of heat show no significant difference from a hypothetical curve representing the sum of the effects of each agent alone. Curves of treatments involving heat and serial dosages of ultraviolet light almost coincide with a hypothetical curve representing ⅞ of the sum of the effects of each alone. There is no evidence that irradiation preceding heat causes an exaggerated injury which application of the same agents in reverse order fails to do, i.e. there is no sensitization to heat through ultraviolet irradiation.

Streptomycin-induced chlorophyll-less races of Euglena. Luigi Provalosi, S. H. Hunter,[1] and Albert Schatz.[2]

Several aseptic clones of *Euglena gracilis*, *E. gracilis* var. *bacillaris* and var. *urophora* became permanently apochlorotic in light through the action of streptomycin. Detailed investigations were made of bleaching as a function of concentration and duration of exposure to streptomycin, sensitivity of proliferating and non-proliferating cells and comparative sensitivity in light and darkness.

The following results were obtained.

There was a smoothly progressive bleaching of the cultures with increasing duration of exposure and increasing concentration of streptomycin.

There was no difference either in the intensity of bleaching between proliferating and non-proliferating cultures or in the time of exposure to streptomycin required, nor between light- and dark-treated cultures under proliferating conditions, in these respects.

There was no evidence of development of streptomycin dependency.

With streptomycin constant at 100 μg/ml., an exposure of 1–8 hours was required for loss of roughly 50 per cent of the color, and 4 or more days for apparently complete bleaching.

With constant time of exposure (15 days), 1 μg/ml. gave roughly 50 per cent loss of color, and bleaching was nearly complete with 40 μg/ml.

Streptomycin did not interfere with the utilization of carbon sources in darkness.

Preliminary microscopic observation of partially bleached mass cultures showed them to consist of both pale and almost fully green individuals. However a few green cells had less thick chloroplasts or only 1–2 ill-defined chloroplasts instead of the usual number. Aged white cells, derived both from primary treated cultures and from subcultures, accumulated an unusually large number of red granules.

The stigma and paramylon grains remained permanently unaltered in all colorless cultures.

The bleached clones have been carried through ten transfers in light without showing any resumption of color.

The development of the basal mat in Hydractinia. Sears Crowell.

Small fragments of the basal mat of Hydractinia to which a few hydranths are attached can be isolated and fed with the nauplii of the brine shrimp, Artemia. These fragments sometimes grow by the extension of stolons. However, in many cases, instead of producing stolons, the edge of the original mat grows out as a whole. Previous descriptions of the mat of Hydractinia state or imply that it arises and grows by the production of stolons which later anastomose

[1] Haskins Laboratories.

[2] Sloan-Kettering Institute for Cancer Research.

and broaden to give a solid mat. It is clear in my specimens that the mat may grow directly at its edge without an intermediate stage of stolon proliferation and subsequent fusion.

Specificity in the fusion of stolons in hydroids. SEARS CROWELL.

The growth and fusion of stolons arising from small fragments of colonies of Hydractinia, Podocoryne, and Stylactis have been observed. When stolons growing out from the same fragment or from separate fragments taken from the same colony meet, they fuse in nearly every instance. However, fusion following contact does not occur between stolons of different genera, of different sex within a species, nor of different colonies of the same species and sex. It is clear that there is individual specificity in the fusion of stolons in these three hydroids.

The distribution of the cerebrospinal fluid in the lower vertebrates. H. P. K. AGERSBORG.[1]

Although a great deal of research has been done in anatomical laboratories in various countries on the distribution and function of the cerebrospinal fluid in some of the higher vertebrates (dog, cat, rabbit, man), the results of this type of research are not generally known among biologists. Since it is to the biologist the physician usually turns for information on basic biological problems, it was thought quite pertinent to tackle a study of this nature in a biological laboratory. It was also thought that if the cerebrospinal fluid is of such great importance in man as the members of the healing arts think, it must be of like importance in not only mammals generally, to which the medical practitioner has turned for experimental enlightenment, but also to the vertebrates as a group of which the mammals including man are a part. For these reasons we have started a study of this problem in the lower vertebrates, using the same methods as have been used by the experimental worker in medical schools on higher vertebrates (mammals), and thinking that if the cerebrospinal fluid is of such great importance to mammals as the members of the healing arts claim, it must be of importance to vertebrates other than mammals, even to the lower vertebrates. If so, we would expect that such a system would be common to all just as the other visceral organs are not only analogous but homologous in all the vertebrates. Homologous organs are such that they are the same in origin and structure. Such is by and large the central nervous system in vertebrates. Therefore, this should hold, regarding the relationship of the nerves and their investments to the central nervous system on the one hand, and to the peripheral terminals on the other. This is exactly what we have established, using elasmobranch and teleost fishes, the frog, and turtle. There is a universal phylogenetic principle prevailing, a principle of common morphology, and perhaps also a common function. To our knowledge, no one has studied this problem in the lower vertebrates before.

Implications of cerebrospinal fluid distribution in the therapy of the healing arts. PAUL E. KIMBERLEY, D.O.[1]

The relationship between the cerebrospinal fluid and the rhythmical pulsations of the brain has been developed to formulate a functional unit. This unit, among other possibilities, aids the movement of cerebrospinal fluid from the intercellular spaces into the perivascular and subarachnoid spaces, thus simulating the mechanisms for moving intercellular fluids in other parts of the body.

The distribution of cerebrospinal fluid, as it has been demonstrated, to the olfactory mucosa, the eye, the inner ear, the visceral nerves including their ganglia and the craniospinal nerves is enhanced and probably motivated by the pulsations of the central nervous system tissue. Thus a factor for promoting the biological requisites of fluid motion has been added which previously was not discussed for the nervous tissues.

The possibility of stasis of cerebrospinal fluid naturally arises when considering the causes for the physiological perversions known as symptoms of disease.

The effect of such a mechanism is seen as a possible factor in the work of investigators using the vertebrates, especially mammals.

[1] Des Moines Still College of Osteopathy and Surgery, Des Moines 9, Iowa.

PAPERS READ BY TITLE

Studies of the chemical form of P^{32} after entry into the arbacia egg. Dr. P. H. Abelson.[1]

Further studies[2] have been made of the P^{32} uptake by eggs of the *Arbacia punctulata*, 1–5 hours after fertilization. When eggs are exposed to sea water containing 10^{-4} to 10^{-5} grams of inorganic phosphate and .02 μc. of P^{32} per ml., an uptake of P^{32} is observed.

The new work shows that at least 97 per cent of the P^{32} appears in the egg as tri-chlor-acetic acid soluble compounds. An attempt was made to determine the chemical nature of these compounds. Procedures outlined by Umbreit[3] et al. were employed yielding barium soluble and barium insoluble fractions. The latter fraction was further divided into an inorganic phosphate component and a precipitate containing ATP and ADP. From hydrolysis studies of the barium soluble material, it is clear that this fraction contains at least two components. Thus one finds at least four chemical forms of P^{32} very soon after entry of the substance into the egg. The total P^{32} increases linearly with time from 1–5 hours after fertilization and the various acid soluble fractions likewise seem to increase linearly.

The difficulties of performing P^{32} chemical analysis in the presence of echinochrome proved enormous. By using small amounts of eggs (.1–.2 cc) and large amounts of carrier, 4 mg. P and 30 mg. ATP, it was possible to obtain more satisfactory separations. The average of twelve determinations performed without ATP carrier gave the following percentages of acid soluble components:

PO_4	ATP & ADP	Ba soluble	Missing
42	23	21	14

With carriers for inorganic phosphate and ATP the results of six determinations gave:

PO_4	ATP & ADP	Ba soluble	Missing
16	41	41	2

It is possible that these results might be altered if carrier for the barium soluble components were available.

Penetration of radioactive phosphate into the eggs of Strongylocentrotus purpuratus, S. franciscanus, and Urechis caupo. S. C. Brooks and E. L. Chambers.[4]

Samples of a 0.2 per cent suspension of (1) unfertilized and (2) fertilized eggs at 15° C., containing P^{32} as orthophosphate at concentrations of 0.001–.30 μc/ml., were centrifuged in Hopkins vaccine tubes. The supernatant was withdrawn and the volume measured to an accuracy of $\sigma = .00015$ ml. The radioactivity of each sample was determined. Correction was made for the activity of the remaining supernatant.

In the unfertilized egg the rate of penetration is slow and remains approximately constant for hours.

In the fertilized egg a definite increase in penetration occurred within the first 6 min. and the rate rapidly rose after 15 min., reaching a maximum by 60 min. (cleavage at 110 min.). After 60 min. the rate remained constant throughout the first three cleavages.

At total initial orthophosphate concentrations of 1.4×10^{-6}, 4.3×10^{-6}, and 2.3×10^{-4} GM/liter the "penetration" constants for the unfertilized eggs were respectively 2.2×10^{-13}, 1.85×10^{-13} and 2.0×10^{-13} GM per ml. of eggs per second while for the fertilized eggs the values were 2.9×10^{-10}, 2.0×10^{-10} and 1.65×10^{-10} GM/ml./sec. In *S. franciscanus* the values at an initial suspension concentration of 1.7×10^{-6} GM/liter were: for the unfertilized eggs, 7.4×10^{-13} GM/ml./sec.;

[1] Carnegie Institute.

[2] Abelson, P. H., *Biological Bulletin*, **93**: 203 (1947).

[3] *Manometric techniques*, Burgess Publishing Co.

[4] Department of Zoology, University of California. Under grant from the N. C. I., U. S. P. N. S.

and for the fertilized, 8.2×10^{-11} GM/ml./sec. Expressing results in terms of GM/ml. of eggs/sec./GM per liter of suspension, the rate of entry into the fertilized eggs of both species was found to be 130–160 times that of the unfertilized.

Continuous washing of fertilized eggs failed to remove more than 2–5 per cent of the contained activity. Therefore, the penetration constants for the fertilized egg essentially represent the rate of increase of total P in the egg, namely, only about 0.1 per cent increase in total P per hour. NaCN at 1×10^{-4} M decreased the rate of penetration threefold.

Eggs of *Urechis caupo* show a similar phenomenon but the increase in rate does not occur until after the second cleavage.

The evidence indicates that (1) the rapid penetration of orthophosphate in the fertilized egg represents an accumulation rather than an exchange; (2) the fraction of orthophosphate entering the fertilized eggs is greater the lower the suspension concentration; (3) during the first three cleavages the rate remains constant irrespective of the mitotic and cleavage cycle, and increase in surface area.

A preliminary account of this work was given at the 28th Annual Meeting of the Pacific Division of the A. A. A. S., San Diego, June 18, 1947.

Distribution of radioactive phosphate in the eggs of the sea urchin Lytechinus pictus. E. L. Chambers, A. Whiteley, R. Chambers and S. C. Brooks.[1]

A single layer of eggs was continuously perfused with sea water containing P^{32} at 0.0005 μc/ml. The eggs were microscopically observed from above, and counting rates taken with a Geiger-Muller tube placed under the chamber. Eggs fertilized in the chamber showed the same uptake curve as described above for *S. purpuratus*.

Samples of membrane-free fertilized eggs in P^{32} sea water were washed in a NaCl/KCl mixture to remove the hyaline plasma layer, and the activity measured. The rate of penetration into these denuded eggs was the same as in the controls.

Eggs containing P^{32} were centrifuged into light and heavy fragments. The equal sized portions of unfertilized eggs showed equal activity per ml. Membrane-less fertilized eggs separate into large, coarse granules and small heavy hyaline portions containing fine granules which stain with methyl green. The activity per ml. of the latter was twice that of the former.

Eggs containing P^{32} were washed and homogenized at different stages of development at 0° C. in 5 per cent trichloracetic acid and the activity of the acid soluble (AS) and acid insoluble (AI) fractions determined. In all cases (unfertilized and fertilized up to 16 celled stage) the activity in the AI fraction varied from 3.5–4.1 per cent of the total. Fertilized eggs exposed to P^{32} from 0′ to 60′, then washed free and homogenized at the blastula stage, showed 6.9 per cent activity in the AI fraction. Activity in the phospholipid fraction of the AI was negligible. Killed eggs homogenized in the presence of P^{32} showed 0.85 per cent of the total activity of the suspension in the AI. This is far below the percentage of activity accumulated in the AI fraction of living eggs.

P^{32} accumulates progressively after fertilization and tends to be more intensely segregated in certain portions. After homogenization in trichloracetic acid the activity is found primarily in the AS fraction. A significant percentage of the activity is found in the AI fraction, which increases during development.

On the combining weight of Cypridina luciferin. Aurin M. Chase.

Giese and Chase (*J. Cell. Comp. Physiol.*, **16**: 237, 1940) calculated a combining weight for partially purified Cypridina luciferin on the basis of its reaction with cyanide. Their value was between 800 and 2400, the lower value seeming more probable. Because of certain sources of error inherent in their method, a redetermination by some other method seemed desirable. The method chosen depends upon the oxidation of luciferin by ferricyanide (Harvey, *J. Biol. Chem.*, **78**: 369, 1928; Anderson, *J. Cell. Comp. Physiol.*, **8**: 261, 1936).

Having established that potassium ferricyanide in low concentrations was without significant effect upon the enzyme, luciferase, the luminescent reaction was measured over a range of ferricyanide concentrations from 10^{-4} to 10^{-9} Molar. The luciferin was from two cycles of puri-

[1] University of California, California Institute of Technology, and M. B. L., Woods Hole, Mass.

fication by Anderson's method (*J. Gen. Physiol.*, **19**: 301, 1935). In one series of experiments the oxidant acted upon the luciferin for one-half minute; in another series, for five minutes. In all cases 0.0016 mg. of the product from the purification procedure was present in 10 ml. of pH 6.70 phosphate buffer containing the desired concentration of ferricyanide.

The percentage of reduced luciferin decreased progressively from 100 per cent, at a ferricyanide concentration of 10^{-4} *M*, to zero per cent at a concentration of about 3×10^{-3} *M*. Using this latter figure and assuming that the luciferin was free of impurity (the degree of purity is not actually known but is probably fairly high) a value of about 500 was calculated for the combining weight. If the oxidation as measured involves a one electron change, which may be the case in the particular procedure used, the molecular weight would be equal to the calculated combining weight, about 500. For a two electron change it would be double this value.

Fat cell size in the mutant small-wings of Habrobracon. A. M. CLARK AND D. S. GROSCH.

The presence of the gene small-wings (sw) reduces the length of wings and the size of wing tissue cells. In order to determine whether this gene also affects other types of cells in the body, measurements of "fat cells" were undertaken. Conditions of measurement as outlined by Grosch (1948—*Proc. N.C. Acad. of Science*) were that the animals be equivalent in size, that they be used immediately upon emergence, and that isosmotic Ringer's solution be used as the suspending fluid. Five animals from each of the six genotypes obtained from $\frac{c\ +}{c\ sw}$ ♀ × + sw ♂ were selected for the present study. The abdomen of each was removed and dissected on a slide containing a drop of Ringer's in order to permit dispersion of the fat cells. Diameters of one hundred fat cells were measured from each animal.

Genotype	$\frac{c}{+}\frac{+}{sw}$ ♂	$\frac{c}{+}\frac{sw}{sw}$ ♂	c + ♂	c sw ♂	$\frac{c}{+}\frac{+}{sw}$ ♀	$\frac{c}{+}\frac{sw}{sw}$ ♀
Mean Fat Cell Diameter ± S.E. in microns	107.36 0.66	103.51 0.66	102.52 0.55	97.57 0.66	98.01 0.66	87.34 0.55

Comparison of diploid males $\left(\frac{c}{+}\frac{+}{sw}\ ♂\right)$ with diploid males $\left(\frac{c}{+}\frac{sw}{sw}\ ♂\right)$, haploids (c + ♂) with haploids (c sw ♂), and females $\left(\frac{c}{+}\frac{+}{sw}\ ♀\right)$ with females $\left(\frac{c}{+}\frac{sw}{sw}\ ♀\right)$ indicates that the mutant small-wings effects smaller fat cells. These size differences are similar in trend to those obtained for the wing cells (Clark and associates—unpublished). Further, comparison of diploid males with haploid males indicates that the diploid males have larger fat cells. This also can be correlated with wing cell size. Comparison of males and females shows a sex difference in that females tend to have smaller fat cells. This sex difference is consistent with findings by Grosch on stock No. 25 and on stock No. 33 which are wild-type except for eye color markers.

The relation of the plasma membrane, vitelline membrane and jelly in the egg of Nereis limbata. DONALD P. COSTELLO.

The relation of the plasma membrane to the extraneous coats and cortical layers of the Nereis egg may be ascertained by observation of the normal living egg and of the egg after certain experimental treatments. Evidence obtained from experiments with the centrifuge and by treating the egg with alkaline sodium chloride indicates that the plasma membrane of the unfertilized egg is external to the jelly precursor granules of the cortex, and just inside the vitelline membrane. Experiments with alkaline sodium chloride (pH 10.5) indicate that the perivitelline space of the fertilized egg is extra-ovular after jelly extrusion is complete. The behavior of the egg in alkaline sodium chloride and the normal cortical response of the egg to

fertilization are attributed largely to the properties of the jelly. Preliminary data (determinations by Ferry, 1939) indicate that it contains less than 1 per cent nitrogen, and is at least 75 per cent carbohydrate (estimated by the Tillmans-Phillipi method, and corrected for ash). The jelly is precipitated in fibrous form by barium ions at alkaline reactions but not at neutrality. The evidence suggests that the jelly is a uronic acid polymer, occurring as the calcium (and perhaps magnesium) salt.

Spiral cleavage is generally assumed to be limited to the polyclad Turbellaria, the Nemertea, the Annelida and all Mollusca except the Cephalopoda. Actually, spiral cleavage is considerably more widespread, if one considers certain modifications of the typical oblique cleavage taking place in four quadrants. In all forms, spiral cleavage becomes modified into bilateral cleavage at some stage in development.

As described by Bresslau (1909, 1928) and Costello (1937), the Acoela, during early cleavage, are characterized by oblique spindles which alternate in their laeotropic and dexiotropic positions to give off three duets of micromeres. The developing egg thus consists of only two hemispheres, in contrast to the four quadrants typical of the Polycladia, Annelida, and Mollusca.

A further deviation is exemplified by Lepas and other Cirripedia. Cleavage of Lepas may be looked upon as a cleavage by "monets," or as unit cleavage. If this unit is designated to correspond to the D-quadrant, three micromeres (1d, 2d, 3d) are given off in succession to give rise to ectoblast and secondary mesoblast, and the fourth micromere given off (4d) is the primary mesoblast. The earliest cleavages have spindles which are definitely oblique. Bigelow (1902) has used the notation of a quadrant system to designate the cleavage products, but he realized that he was not dealing with a four-quadrant system. This was in disagreement with the comments of Mark and Castle, who considered Lepas to have a slightly modified four-quadrant cleavage.

Induction of autogamy in single animals of Paramecium calkinsi following mixture of two mating types. WILLIAM F. DILLER.

Mixtures of two mating types of *P. calkinsi*, obtained through the courtesy of C. B. Metz, give rise to autogamous reorganization in single animals, in addition to normal conjugating pairs and "pseudo-selfing" clumps of a number of reorganizing individuals, loosely joined together. The latter are presumably similar to those resulting from mixture of living animals and dead animals of the opposite mating type of *P. aurelia* (Metz, '47). This race of *P. calkinsi* is characterized by a single micronucleus. A fungoid symbiont of the macronucleus is frequently found. Nuclear activity in autogamy in single animals of *P. calkinsi* closely resembles the nuclear phenomena of conjugation and both are much like the corresponding processes in *P. aurelia*. Typical crescent stages are rarely, if ever, found in single autogamous individuals. This is very different from the conditions in *P. aurelia* (Diller, '36) and *P. polycaryum* (Diller, unpublished data). Large swollen first meiotic prophase nuclei are often seen. Each of the two young macronuclear anlagen in both ex-conjugants and autogamous animals contains about thirty deeply staining granules which are probably enlarged, modified chromosomes. This point is being investigated further.

An extra post-zygotic division in Paramecium caudatum. WILLIAM F. DILLER.

Three post-zygotic divisions following fertilization have been considered the normal procedure in the conjugation of *P. caudatum*. In a race from a Philadelphia pond in which the conjugants separate normally in a late stage of the division of the synkaryon, evidence has been secured indicating that four post-zygotic divisions occur, though only one of the nuclear products of the first division takes part in all of them. One of these sister nuclei degenerates, while the other divides three additional times to produce eight nuclei, four of which become macronuclear anlagen. This extra division, with degeneration after the first, agrees with Chen's recent findings in *P. bursaria*.

Further observations on the metabolism of clams' tissues in sea water at different salinities. HOYT S. HOPKINS.

Earlier results (*Anat. Record*, **96**: 522, 1946) indicated that the stimulating effect of diluted sea water upon oxygen consumption of excised gill tissue of *Venus mercenaria* was not primarily

due to reduced concentration of any specific cation of sea water. The higher rate of respiration in ⅔ sea water was maintained when the concentration of K or Ca was restored to that in natural sea water, without raising the osmotic pressure. Replacement of Mg salts caused a reduction of one-third in the respiratory increment above that in normal sea water.

Recent experiments with Van't Hoff's solution led to a similar conclusion: that the dilution effect is not the result of a reduction in concentration of particular salts. In this artificial medium the oxygen consumption of gill was slightly lower (9 per cent) than in natural sea water. The rate increased over 40 per cent in Van't Hoff's solution diluted to ⅔, which was about as much as in diluted natural sea water. When Mg salts were omitted from the solution, maintaining the same osmotic pressure, there was an increase in oxygen consumption of about 20 per cent. If half of the Mg was added—giving a concentration approximating that in the diluted sea water experiments—the rise in oxygen consumption was negligible. Since the respiration of gill was augmented in diluted sea water, but not when isotonic NaCl, sucrose, or glucose solution replaced water as the diluent, osmotic work may have been involved in the first case. Also favoring an osmotic interpretation is the finding that superficial tissues (gill, mantle) showed increased respiration in dilute sea water, whereas muscle showed a decrease.

The stimulated respiration in hypotonic sea water, like that in isotonic NaCl, may involve hydration, since slightly macerated gill showed equally high rates in normal and diluted sea water.

Studies on the red blood cells of fish. Dr. Bruno Kisch.

In this paper investigations on erythrocytes of fish are reported. The hemoglobin content, red blood count, and size of the red blood cells have been measured in different species of Teleostae and Selachians. The investigations were performed in August 1948. The hemoglobin content was determined with a Leitz Haemoglobinometer with colored glass rods as standards for comparison (Sahli's method). The blood count was taken by the usual routine method. The size of the erythrocytes was measured by an ocular micrometer.

Teleosts

Name	No. of animals investigated	Hemoglobin per 1 cc.	Red blood count in 1 mm.³	Diameter in μ of red blood cells
1. Puffer	2	7.35	4,410,000	8.7 : 6.9
2. Scup	1	10.6	3,990,000	8.4 : 6.9
3. Tautog	3	6.7	3,350,000	10.9 : 7.2
4. Porgy	4	13.4	3,170,000	9.8 : 7.2
5. Sea Robin	2	7.1	2,380,000	10.0 : 6.3

Selachians

Name	No. of animals investigated	Hemoglobin per 1 cc.	Red blood count in 1 mm.³	Diameter in μ of red blood cells
1. Mustelus canis	5	3.92	452,000	16.3 : 10.9
2. Carcharinas obsc	1	3.5	540,000	19.0 : 13.5
3. Raja eglant.	2	4.8	320,000	24.3 : 14.2
4. Raja stabilifor.	3	3.6	260,000	21.3 : 14.5

These figures show: Teleosts have a hemoglobin content of the blood 2 to 3 times higher than Selachians and a red blood count which is 5–8 times as high as that of Selachians, but the size of their erythrocytes is about ½ that of the elasmobranch fish.

Among the latter a definite difference has been found between the quick sharks and the slow skates, not so much concerning the hemoglobin content as concerning the red blood cells.

The red blood cells of two Mustelus embryos were much bigger than those of the grown

animals, rather rounder than elliptic, and the nuclei showed a chromosome filament. The nuclei were less compact and bigger than those of grown animals.

Among the teleosts, the swordfish showed small blood cells about the size of those of a puffer. The biggest erythrocytes among the bone fish, very similar to those of the elasmobranch fish, were found in a specimen of the plectognate Mola Mola; they were 12.5 : 9.4 in size.

It is the impression of the author that the relation between size and number of red blood cells in fish is only a single instance of a general rule in biology.

Whenever the same function is accomplished in different species, either by a few or by many functioning units, the size of each of the few units is big whereas the size of each of the many units is small. The set-up with the many small units seems to be the more efficient one, the set-up with the few big units the more primitive, less efficient one.

It seems to be similar with the size and number of the glomeruli of the kidney for instance in the murida family and among the Chinoptera as can be judged by the data given by Denzer.

Dr. R. Chambers was kind enough to call my attention to the chromosomes of different species of the family Cyclops where one species (*Cyclops brevispinosus*) has four very big and another (*C. viridis*) twelve very small chromosomes.

Further studies will show how far this rule applies to nerve ganglions and other functioning units of the living beings.

The action of NH_4Cl on the surface membranes of Arbacia eggs. M. J. KOPAC.

A new granular layer can be revealed on centrifugation of unfertilized or fertilized Arbacia eggs immersed for several hours in 0.53 M NH_4Cl (3 parts) + sea water (7 parts). This layer, less dense than the cytoplasmic matrix, is believed to contain released lipids (Heilbrunn, *Biol. Bull.*, **71**: 299, 1936). White halves, obtained by centrifugal splitting of unfertilized eggs, when treated with NH_4Cl showed almost as much "lipid" substance as whole eggs. Much of this material, therefore, originates in the cytoplasmic matrix.

Unfertilized Arbacia eggs, after treatment with NH_4Cl, were tested by the oil coalescency method (Kopac, *Cold Spring Harbor Symposia*, **8**: 154, 1940). Following 90 to 120 minutes' immersion, the eggs readily coalesced with oil drops indicating the disappearance of jelly. The coalescency of the treated eggs progressively increased the longer the eggs were exposed to NH_4Cl, thereby indicating a weakening of the vitelline membrane. This was further suggested by the flimsy fertilization membranes that developed after insemination.

Unfertilized eggs, after 4 to 6 hours' exposure to NH_4Cl, were stratified by centrifugation. Oil drops were applied to the surface adjacent to the lipid, matrix, yolk, and pigment zones where coalescence occurred readily. Immediately after coalescence near the matrix zone, a wave of peripheral disintegration encompassed the entire cell surface resulting in complete cytolysis of the cell. On occasion, this effect was obtained at the lipid zone. No cytolysis occurred on coalescence at the pigment zone nor at junction of pigment and yolk zones.

Red halves from centrifuged, NH_4Cl-treated cells sometimes retained a small portion of the matrix. Coalescence with oil drops at this region also induced the peripheral wave of disintegration. No cytolysis occurred when yolk or pigment zones were similarly treated.

A similar peripheral disintegration following coalescence was previously observed in completely denuded eggs. This reaction always occurs when extraneous coats are not present to lend their mechanical support to the underlying protoplasmic surface layer. The absence of peripheral disintegration when oil drops penetrated near the pigment of yolk zones suggests a new role for packed cell inclusions. The packed granules and vacuoles can serve as an inner mechanical support for the protoplasmic surface layer, thus replacing the extraneous coats.

These data suggest that, in addition to releasing bound cytoplasmic lipids, NH_4Cl may alter the surfaces of Arbacia eggs. The principal action, however, appears to be on extraneous coats, e.g., jelly, vitelline membrane, and hyaline layer.

The inhibition of development of Arbacia eggs by NH_4Cl. M. J. KOPAC.

Immersion of unfertilized Arbacia eggs in 0.53 M NH_4Cl (3 parts) + sea water (7 parts) for several hours altered, inter alia, the density ratio between cytoplasmic matrix and nucleus. Following centrifugation of treated eggs, the nucleus assumed an equilibrium position near the yolk boundary rather than near the oil cap as in normal eggs.

Nearly all cells immersed in NH_4Cl-sea water up to 4 hours prior to insemination cleaved although there was considerable delay. About 50 per cent of the fertilized eggs divided following 7 hours' exposure. None divided following 13 hours' exposure. Before insemination, the cells were transferred to sea water.

Subsequent development in normal sea water was slow and irregular. Following 2 to 3 hours' exposure to NH_4Cl, 50 per cent of the eggs developed into swimming blastulae. Following 5 to 6 hours' exposure, 10 per cent formed swimming blastulae. Gastrulation was completely inhibited by only 15 minutes' exposure. The blastulae were sluggish and swam in tight circles near the bottom of the vessel.

White and red halves obtained by centrifugal splitting of unfertilized eggs, previously exposed to NH_4Cl for 4 hours, also cleaved following insemination. The red halves cleaved more rapidly than the white halves which contained all the displaceable low density substances. Development was irregular and only a few of the red halves developed beyond the 12- or 16-cell stages.

Fertilization membranes were weak and many ruptured as early as the 2- or 4-cell stage. Double and triple blastulae were common. Frequently, one part of the double blastula was well developed while the other part consisted of disorganized blastomeres which rarely held together. Some of these blastomeres had no hyaline layer as revealed by the oil coalescency method.

Several examples were noted where one blastula, the size of the first blastomere, developed normally while attached to it were 2, 4, or more cells, apparently arising from the second blastomere formed during the first cleavage.

The weakened extraneous coats are probably responsible for this irregular development. The fertilization membranes were weak and these did not elevate from the cell surface more than 2–3 microns. The hyaline layer was variable in thickness even during the first or second cleavages. Later, it frequently became so weak that bizarre aggregates of cells were produced instead of organized blastulae.

The nature of the hemolytic effect of silver. MARIAN E. LEFEVRE AND M. H. JACOBS.

The hemolysis of certain fish erythrocytes by an impurity in some brands of C. P. NaCl, shown by Ball to be silver, has been further studied, using chiefly the erythrocytes of two sensitive species, the mackerel and the cunner. It has been determined that the mechanism of this hemolytic effect is an induced permeability of the erythrocytes to cations resulting in a swelling of the cells by the operation of the Donnan equilibrium. The evidence is: (1) that immediate and extensive shrinkage of the cells occurs in sucrose made isosmotic with the blood and containing a trace of calcium, (2) that swelling and hemolysis are rapid in NaCl of any strength, (3) that on the addition of concentrated NaCl to a suspension of cells in an appropriate mixture of NaCl and sucrose there is an immediate shrinkage, quickly followed by a return to more than the original volume, (4) that hemolysis in NaCl is prevented by the addition of relatively low concentrations of sucrose, provided that Ca is present, and, (5) that under properly chosen conditions the rate of hemolysis after treatment with silver shows a minimum at a pH near the isoelectric point of hemoglobin.

In the course of this work it was noted that the erythrocytes of the two species of fish in question, and some others, undergo hemolysis within an hour or less in pure isosmotic sucrose solutions. As in the case of the much less striking injury of mammalian erythrocytes in the non-electrolyte solutions studied by Wildbrandt, hemolysis of the fish erythrocytes was only feebly opposed by NaCl but very effectively by low concentrations of $CaCl_2$, $BaCl_2$, or $MgCl_2$.

It was also noted that the well-washed erythrocytes of several species of fish, unlike mammalian erythrocytes, may fail to hemolyze within several hours in a pure isosmotic solution of NH_4Cl. On the addition of a trace of bicarbonate, however, hemolysis in the same solution readily occurs.

Reversible sphering of erythrocytes. WARNER E. LOVE AND M. H. JACOBS.

The sphering action on human erythrocytes of sodium taurocholate and the fact that it is favored by alkalinity are well known. It has apparently not previously been reported, however, that sphering of taurocholate treated cells can be produced and reversed at will many times in succession merely by appropriate changes in the pH of the surrounding medium.

A convenient way of showing this effect is to suspend well washed human erythrocytes in an isotonic NaCl solution buffered about neutrality with phosphate and lightly colored with phenol red, which in low concentrations has little effect on the cells. With a little NaOH the indicator is now made distinctly red and taurocholate is added until sphering just occurs. On the addition of HCl until the indicator becomes yellow the cells immediately resume their biconcave shape. In the absence of phenol red the behavior of the cells themselves serves as a sensitive indicator of pH changes.

Alkali and acid may now be added alternately as often as desired. We have in this way produced sphering and its reversal 15 times in succession. If only enough taurocholate be used to cause sphering, the cell may remain in good condition and be capable of showing reversal of sphering for at least 24 hours. Several washings of the taurocholate-treated cells with isotonic NaCl do not destroy their ability to show the behavior just described. Under favorable conditions a pH change of 0.5 unit or less is sufficient to reverse the taurocholate effect, but even much larger changes have little influence on the sphering produced by sodium cetyl sulfate or sodium cetyl phosphate.

Observations on the hemolytic effect of sodium dodecyl sulfate. LOIS H. LOVE AND M. H. JACOBS.

The hemolysis of human erythrocytes by sodium dodecyl sulfate has several unusual aspects. The first is the effect of temperature on the rate of the process. At low temperatures with some concentrations complete hemolysis occurs within a few seconds, but as the temperature is increased, a point is reached where the process suddenly becomes very slow. Under some conditions a rise of temperature of 1° C. may change the time for complete hemolysis from less than a minute to more than an hour. With a further rise of temperature the rate of hemolysis may again become more rapid.

In the temperature range of retardation two phases of hemolysis can be demonstrated, some of the cells hemolyzing immediately, the remainder very slowly. The slowly hemolyzing cells, however, show a normal susceptibility to the hemolytic action of hypotonic solutions.

The second peculiarity of hemolysis by sodium dodecyl sulfate is that the addition of lecithin causes a remarkable acceleration of the process instead of the retardation reported in the case of certain other hemolytic agents. For example, in one experiment 80 per cent hemolysis by sodium dodecyl sulfate alone required 160 minutes; in the presence of approximately 50 γ of lecithin alone no hemolysis occurred in 180 minutes. But in a mixture of the two the time for 80 per cent hemolysis was 16 minutes. By a proper choice of conditions even an apparently non-hemolytic concentration of sodium dodecyl sulfate can be made hemolytic by addition of a non-hemolytic concentration of lecithin.

Effects of hypertonic solutions on Nereis eggs. W. J. V. OSTERHOUT.

Unfertilized eggs of *Nereis limbata* placed in 1.4 M $MgSO_4$ or in 1.5 M dextrose in sea water lose water rapidly and appear collapsed with abnormal shapes. If after a few minutes they are replaced in sea water, they soon become normal in size and appearance. If sperm is promptly added many eggs extrude jelly, segment and develop trochophores.

If the unfertilized eggs are placed in 2.7 M $MgSO_4$ in sea water, they collapse at once, but in the course of time $MgSO_4$ enters the eggs sufficiently to enable them to resume more nearly normal shape. The same thing happens in 2.8 M dextrose in sea water but here the penetration is much more rapid.

When the unfertilized eggs are exposed for a short period to 2.7 M $MgSO_4$ in sea water and are returned to sea water they swell rapidly and develop a clear zone just beneath the vitelline membrane. Such eggs cannot extrude jelly nor segment nor produce trochophores when sperm is added.

Solubility of the vitelline membrane of Nereis eggs. W. J. V. OSTERHOUT.

The vitelline membrane of Nereis resists acid and alkali and all other reagents hitherto tried. But it dissolves quickly in a mixture of sodium dodecyl and tetradecyl sulfate. When unfertilized eggs are placed in 1 per cent of this reagent in sea water the egg swells and soon shows a

protrusion at one point as though the tough vitelline membrane were yielding to internal pressure.

The membrane covering the end of the protrusion dissolves and this action spreads to surrounding regions and in a few minutes the membrane completely disappears. A similar process takes place in segmenting eggs.

The dissolving of the vitelline membrane is more rapid and more easily observed if the eggs are first killed by placing them for a few minutes in 2.7 M $MgSO_4$ in sea water and then leaving them for a short time in sea water before they go into the dissolving reagent. In sea water they swell and acquire a clear zone inside the vitelline membrane. In the reagent splits appear at several places in the membrane and at these spots the membrane dissolves and this action spreads to neighboring regions so that the membrane completely disappears. This process is accompanied by alterations in the appearance of the protoplasm.

Experiments on chloroplasts and on photosynthesis. W. J. V. Osterhout.

The chloroplasts of certain marine and fresh water algae and of some other plants show very interesting reactions when the cells are bathed in a saturated solution of hexylresorcinol. The chloroplasts shrink and clump together and in many cases form a more or less regular network. This continues to contract and may eventually form a single compact mass occupying only a small portion of the cell.

There is great loss of water from the chloroplast and the minute drops of chlorophyll (grana) originally present in the chloroplast are evidently brought closer together and it seems possible that they may fuse to some extent.

Chloroplasts exposed to "Sodium Lorol Sulfate" (which may be called S. L. S. for convenience) also show great contraction. This reagent is a mixture of sodium dodecyl and tetradecyl sulfate. When cells of *Spirogyra* are placed in 1 per cent S. L. S. in distilled water, the chloroplast contracts and eventually breaks up into small masses. The green color soon spreads uniformly throughout the cell as if in aqueous solution but is unable to pass out through the cellulose wall. Cells in this condition show no photosynthesis when well washed and placed in a solution of sodium bicarbonate with a trace of phenolphthalein as indicator. But cells subjected for 2 minutes to 0.01 per cent S. L. S. in which the chloroplast is still intact show photosynthesis though slightly delayed as compared with the control. When the cells are exposed for 8 minutes to the same reagent, the cells at first appear not to be seriously affected, but on washing thoroughly and transferring them to the bicarbonate solution containing phenolphthalein, the injury progresses until the chloroplasts are affected. Such cells show no photosynthesis within 1 hour or longer. The control cells show rapid photosynthesis.

A new peritrich from Woods Hole. M. A. Rudzinska.

Pachystomus Olistus (cf. Abstract Section *Biological Bulletin*, **93**: 2, 1947).

Inter-myotome connections in early embryos of Mustelis canis. Lois E. TeWinkel.

Embryos of the smooth dogfish, *Mustelis canis*, begin to flex rhythmically from side to side at an approximate length of 3.5 mm. (20–23 somites). Such motion, observed in a number of elasmobranch embryos and shown by Wintrebert to be aneural, is exhibited for some time prior to the initiation of the heart beat, which, in the Mustelis embryos studied, occurred at the 5.5–6 mm. stage.

Sections in the three usual planes show that the antero-posterior orientation of myoblasts has occurred in at least the anterior two-thirds of the somites in 3.5–4 mm. embryos. Cell boundaries are virtually impossible to distinguish but preliminary studies indicate that myoblasts of a given somite come into contact with those of immediately adjacent somites so that there is an interdigitation of their tapering ends. Fibrillar structures seen in the cells are identified tentatively as early myofibrillae. In general they are homogeneous but occasional faint signs of beading suggest the development of cross striations. In 5.8 and 8 mm. embryos, myoblasts of one myotome seem to be directly connected to those of neighboring myotomes at a more constricted area. Distinctly striated myofibrillae, which are now present, appear to be joined to those in cells of adjacent myotomes by means of fine light-staining homogeneous fibrils which extend across the inter-myotome bridge.

At the 35 mm. stage, additional bundles of striated muscle cells have differentiated appar-

ently from mesenchyme cells between the myotome bands and the notochord, but these bundles are separated inter-segmentally by numerous mesenchyme cells. The contrast, therefore, between the longitudinally connected primary myotome bands and the segmented muscle bundles which develop later suggests that there may well be some relationship between myotome bridges and the rhythmic flexion of the early embryo.

Properties of the surface coat in embryos of Fundulus heteroclitus. J. P. TRINKAUS.

The eggs of Fundulus possess a surface layer similar to the surface coat of amphibian eggs. This coat constitutes the outer layer of the egg in all stages examined (immature, mature eggs, and developmental stages through blastopore closure). Microdissection demonstrates it to be an elastic gel, non-adhesive on the outer surface, sticky and less viscous at deeper levels. It solates in Ca-free SW and upon mechanical agitation.

A wound in the coat covering the yolk first widens and then closes in 2–5 minutes (even though yolk is exuded throughout the process). During wound closure, radiating folds appear in the coat. When carbon particles are placed on opposite edges of a closing wound they move toward each other, indicating that wound closure is not due to the formation of a new surface, but to the expansion of an already present, elastic coat. Closure of a wound in the yolk coat near the marginal periblast apparently exerts tension, which not only stretches adjacent cells greatly, but also pulls a large sector of blastoderm toward the wounded area. A wound in the blastoderm itself initiates similar processes of wound healing, resulting in radial stretching of epiblast cells toward the wound closure.

Carbon marking of normal cleavage and gastrula stages suggests a spreading of the surface layer in both cleavage and epiboly. At closure of blastopore of normal embryos, the cells at the blastopore lips elongate greatly, as if they are being pulled toward the point of blastopore closure. At an earlier stage (Oppenheimer, '14), this phenomenon can be simulated and blastopore closure hastened, if the yolk coat is wounded at the point of future closure of blastopore.

These preliminary studies suggest that the surface coat of the Fundulus egg, because of its remarkable elastic properties, plays a unifying and perhaps a causal role in gastrulation movements.

Fertilizin of Nereis limbata. ALBERT TYLER.

The fertilizins of eggs of sea-urchins and other marine animals are known to comprise the material of the gelatinous coat of the unfertilized egg. Since, in Nereis, a gelatinous coat forms after fertilization it is of interest to learn whether or not the material of this coat is fertilizin and to determine some of its chemical properties. Using the alkaline saline method of Costello (1945) for removal of the membrane, and extrusion and dissolution of the Nereis egg jelly, large quantities of this material were prepared with very little injury to the eggs. When added to sperm, under proper conditions of pH (ca. 9) the preparations gave strong agglutination, the titers being considerably greater than those given by ordinary egg water prepared from unfertilized egg in the same time interval. While definitive proof is yet to be obtained, various tests favor the view that the jelly material is fertilizin and that that present in ordinary egg water is similar material that has diffused through the membrane in non-gelatinous form. The active material is found to be non-dialyzable, fairly heat stable and precipitable by 60 per cent alcohol. It has a nitrogen content of about 5 per cent and contains at least 18 per cent reducing sugar. From a total of approximately 20 cc. of eggs that have been obtained so far in the present season, the yield of alcohol precipitable material amounts to 300 mg., most of which is available for further analysis.

Mating types and conjugation of four different races of Paramecium calkinsi and the effect of x-rays on the mating reaction. RALPH WICHTERMAN.

Four different races of *Paramecium calkinsi* have been cultivated up to two years in a medium consisting of 2 parts of lettuce infusion and one part of filtered sea-water. Opposite mating types I and II (Yale races) are unimicronucleate; the remaining strains here designated as IIa and IIb are unimicronucleate and bimicronucleate respectively as shown in Hematoxylin and Feulgen preparations. Micronuclei in all races are small and average only 2 μ. Races IIa and bimicronucleate IIb readily mate and conjugate with unimicronucleate type I. All races

appear to be as stable as *P. bursaria* since no change in mating type has occurred during the period in culture. In all cases, the mating reaction occurs with the formation of clumps of a 100 or more specimens any time of day, followed by conjugation. Cultures containing *P. calkinsi* have, at approximately 24° C., a pH range of 6.5 at time of inoculation to pH 7.8 in older cultures. Greatest numbers of paramecia reactive for mating are found in cultures with a pH of 7.3 usually on the 7th day after inoculation.

P. calkinsi is much more sensitive to x-rays than *P. bursaria*. When opposite mating types are irradiated with 100,000 r, 200,000 r and 300,000 r and mixed, the mating reaction occurs but the clumps thus formed are progressively smaller than the controls. Clumps then break down leaving only single specimens. However, mating types irradiated with 100,000 r and mixed will, 24–48 hours later, demonstrate pairs in conjugation. Those irradiated with 200,000 r and 300,000 r will demonstrate the mating reaction but do not enter into conjugation with the exception of some survivors of 200,000 r dosage. When irradiated with 400,000 r and mixed, the slowly moving specimens do not show the mating reaction but die within a few hours. All unirradiated members of one sex type show the mating reaction with specimens of opposite type irradiated up to 400,000 r. Only those irradiated with 100,000 r and some survivors of 200,000 r will conjugate with unirradiated specimens of opposite type. Unirradiated specimens of one sex type will not mate or conjugate with irradiation-killed specimens of opposite sex type.

The hydrogen-ion concentration in the cultivation and growth of eight species of Paramecium. RALPH WICHTERMAN.

A study was made of the pH changes occurring daily in 36 cultures of races of eight fresh and brackish-water species of Paramecium. Original cultures have been maintained continuously for 1–13 years in covered 250 ml. flasks with little if any variation in culture technique. *P. aurelia, P. caudatum, P. multimicronucleatum* and *P. trichium* have been cultivated in hay medium consisting of 1½ gm. of hay and 210 ml. of boiled distilled water which prior to inoculation had a pH of 6.2; *P. bursaria* and *P. polycaryum* in lettuce medium consisting of 1½ gm. desiccated lettuce to 1 liter of boiled distilled water which was filtered then autoclaved and had a pH of 5.0 prior to inoculation; *P. calkinsi* and *P. woodruffi* on lettuce-sea-water medium with a ratio of 2 : 1 respectively and a pH of 6.5 before inoculation.

All media were prepared on one day and inoculated the following day with approximately 20 ml. from a rich culture containing paramecia and bacteria. Daily pH determinations, which in the course of the investigation totalled over 500, were made of the medium prior to inoculation, through the period of maximal growth and gradual decline of the population at about 24° C. with a Cambridge electronic pH meter having a sensitivity of 0.02 pH unit. Buffers were not added to cultures. Results are briefly summarized in the table.

Species	Medium and pH before inoculation	pH range of medium in cultures	Optimum range in which growth occurred and yielded greatest concentration of paramecia
P. aurelia	Hay: 6.2	6.2–7.3	7.0–7.2
P. caudatum	Hay: 6.2	6.2–7.2	6.9–7.1
P. multimicronucleatum	Hay: 6.2	6.2–7.5	6.5–7.0
P. trichium	Hay: 6.2	6.2–7.1	6.7–7.1
P. bursaria	Lettuce: 5.0	5.0–7.4	7.1–7.3
P. polycaryum	Lettuce: 5.0	5.0–7.5	6.9–7.3
P. calkinsi	Lettuce-sea H_2O: 6.5	6.5–7.8	7.1–7.4
P. woodruffi	Lettuce-sea H_2O: 6.5	6.5–7.5	7.0–7.5

The cage hypothesis and a common feature of X-ray diffraction studies of crystalline proteins. DOROTHY WRINCH.[1]

It is well recognized that X-ray diffraction data obtained from protein crystals constitute—potentially at least—crucial tests for any proposed theory of the atomic patterns of proteins.

[1] Smith College.

Fundamental difficulties however arise in using these data for this purpose in any detailed manner in that (1) a protein unit is in general an array of molecules, (2) a protein molecule comprises a skeleton plus a complement of substituents in most cases incompletely characterized and in all cases unknown as regards their spatial pattern, (3) protein crystals contain large numbers of "foreign" molecules and ions, (4) the diffraction patterns to be expected from vast arrays of atoms present scientific and technical problems of considerable complexity (Wrinch, Fourier transforms and structure factors, Am. Soc. for X-Ray and Electron Diffraction, 1946).

In a hypothesis formulated in 1936 (Wrinch, *Proc. Roy. Soc. London* **A161**: 505, 1937), protein molecules were formulated as cage structures, space-enclosing networks of multiply-connected α-levo amino acid backbones, with R-substituents emerging from C_α atoms. Naturally it has, as yet, not proved possible to allocate particular substituents to particular C_α sites, nor even to formulate the numbers or the spatial patterns of the molecules in various protein particles. The fact that this hypothesis gives a skeletal structure which is cubic leads to a picture of protein particles with molecular patterns in which the orientations of the skeletons are structurally related, and thus to the possibility that the constructional principle common to proteins may prove, in some measure, recognizable in diffraction patterns, notwithstanding the fact that the skeletal atoms may constitute a third or less of the atoms in the crystal.

This communication reports the fact that the series of cage structures, C_1, C_2, . . . C_n, . . . have one common feature in their diffraction patterns. The interaction of one pair of antipodal tetrahedral faces of the C_n structure yields maxima in diffraction space, at distances 8 an (1, 1/2, 1/3, . . . $1/n$, . . .) A from the origin, where a—say $\backsim$1.5A—is the mean of the N–C_α, C_α–C, C–N bond lengths. Thus, for the whole series, these interactions produce a maximum at $\backsim$12A. The remaining three pairs of antipodal faces modify the maximum but slightly, moving them from $\sim$12A to $\sim$10A in C_1, from $\sim$12A to $\backsim$11.2A in C_2, from $\backsim$12A to $\backsim$11.6A in C_3, and so on. When the closely packed substituents on the cage faces are taken into account, these maxima may move to slightly shorter spacings (loc. cit., p. 55).

It is well known that crystalline proteins as a class yield strong spacings in the neighborhood of 10–11.5A. It may therefore be claimed that the cage hypothesis meets the first challenge of the X-ray data in that it predicts one or more of a series of structures, all of which have pairs of antipodal faces which, with the remaining faces, produce high intensities at distances of this order of magnitude. It is pertinent to notice that the maxima derived from the predicted skeletons lie in octettes at the corners of a cube and that in the case of tobacco mosaic virus strong spacings at $\backsim$11.3A, close to cube corners, have been recorded (Bernal and Fankuchen, *J. Gen. Physiol.*, **25**: 111, 1941).

(This work is supported by the Office of Naval Research under contract N8onr-579.)

REPORT ON THE LALOR FELLOWSHIP RESEARCH

Phosphagen in annelids (*Polychaeta*). ERNEST BALDWIN * AND WARREN H. YUDKIN.

Kutscher and his school came to the conclusion, upheld by Andrew Hunter, that the creatine which is characteristic of vertebrate muscle is replaced by arginine in the muscles of invertebrates. The discovery of creatine phosphate in the muscle of vertebrates by Eggleton and Eggleton (*Biochem. J.*, **21**: 185, 1927) and Fiske and Subbarow (*J. Biol. Chem.*, **81**: 629, 1929) was shortly followed by that of arginine phosphate in invertebrates by Meyerhof and Lohmann (*Biochem. Ztschr.*, **196**: 22, 49, 1928). Comparative investigations by the Eggletons (*J. Physiol.*, **65**: 15, 1928) and by Meyerhof (*Arch. di Sci. Biol.*, **12**: 536, 1928) supported the broad principle of alternative occurrence. The wider comparative studies of Needham, Needham, Baldwin, and J. Yudkin (*Proc. Roy. Soc. London B*, **110**: 260, 1932) upheld this general principle, but with certain notable exceptions: both phosphagens were found side by side in the body muscles of *Balanoglossus* and in the jaw muscles of an echinoid. These results provided chemical support for the echinoderm-enteropneust theory of vertebrate ancestry proposed by Bateson on morphological grounds. Our present investigation has demonstrated that the enteropneust, *Saccoglossus*, contains only the single phophagen, creatine phosphate.

* Lalor Fellow.

One of the objects of our present study was to investigate the phosphagen of annelids more carefully than has hitherto been done for, while Meyerhof and Needham, Needham, Baldwin and J. Yudkin alike had demonstrated the presence in polychaetes and gephyreans of a labile substance having the general properties of arginine phosphate, Arnold and Luck (*J. Biol. Chem.*, **99**: 677, 1933) were unable to find evidence for the presence of arginine itself in any of the marine worms they studied, though arginine was found in the terrestrial oligochaete *Lumbricus* and has in fact been isolated from this form by Kutscher and Ackermann (*Ztschr. physiol. Chem.* **199**: 266, 1931). Moreover, in the original experiments of Needham, Needham, Baldwin, and J. Yudkin the phosphagen of *Nereis diversicolor* showed somewhat anomalous behavior which was not further investigated at that time. Recently, Greenwald (*J. Biol. Chem.*, **162**: 239, 1941), employing Jaffe's reaction, demonstrated the presence of chromogenic material indicative of creatine in the testes of several annelids.

In the present investigation experiments were done on the atypical behavior of *Neanthes* phosphagen upon hydrolysis in the presence and the absence of the molybdate ion. These experiments showed the presence in this annelid of a labile phosphate compound having the properties of creatine phosphate, together with a phosphagen similar in behavior to arginine phosphate, which for the present we propose to call the "annelid phosphagen." Studies of other polychaetous annelids have now shown that the apparent creatine phosphate and the annelid phosphagen may occur singly or together. From Table I we may generalize concerning the species examined: All the free swimming forms contain creatine phosphate, sometimes together with the annelid phosphagen; all the sedentary forms, with the exception of *Chaetopterus*, contain the annelid phosphagen, sometimes together with creatine phosphate.

TABLE I

Occurrence of phosphagen in polychaetes

The annelid phosphagen similar to arginine phosphate is signified by AP. CP indicates the phosphagen behaving like creatine phosphate.

	AP	CP
Free Swimming Forms (Errantia)		
Orbinia (*Aricia*)	−	+
Diopatra	−	+
Lumbrineris (*Lumbrinereis*)	−	+
Arabella	−	+
Glycera	−	+
Neanthes (*Nereis*)	+	+
Lepidometria	+	+
Sthenelais	+	+
Sedentary Forms (Sedentaria)		
Chaetopterus	−	+
Cistenides	+	+
Amphitrite	+	−
Pista	+	−
Enoplobranchus	+	−
Cirratulus	+	−
Maldane	+	−
Clymenella	+	−

These newer observations show that Needham, Needham, Baldwin, and J. Yudkin's former conclusions must be seriously modified. Whether they must actually be abandoned will depend on the results of further, thoroughgoing investigations of other annelid groups and of more representatives of other invertebrate phyla.

We are pleased to acknowledge our indebtedness to Dr. Frank A. Brown, Jr., for procuring and identifying most of the annelids used in this study.

The mechanism of interaction of inhibitors with human plasma cholinesterase. AVRAM GOLDSTEIN.*

The carbamic esters prostigmine, physostigmine, and carbaminoylcholine inhibit purified human plasma cholinesterase activity as determined by addition of acetylcholine to an enzyme-inhibitor mixture. During a period of two hours after addition of substrate there is a progressive relief of inhibition which is not the result of destruction of the inhibitor. Thus acetylcholine *slowly* displaces these inhibitors from combination with the enzyme. The dissociation that occurs on dilution of an enzyme-inhibitor mixture is also slow, requiring two to three hours for completon. These compounds are reversible inhibitors (by dialysis). Determination of the nature of inhibition by varying substrate concentration by the method of Lineweaver and Burk (*J. A. C. S.*, **56**: 658, 1934) reveals an apparent non-competitive inhibition, i.e., in a twenty minute period of determination no amount of substrate can reverse the combination. If, however, substrate is given full access to the enzyme (by its addition simultaneously with inhibitor) completely competitive curves are found. . . . In contrast to the above, choline, acetyl-b-methyl choline, procaine, methylene blue and other reversible inhibitors are competitive in the varying substrate experiments, but their displacement by substrate proceeds very rapidly. . . . A curious paradox is presented by mercuric ion (chloride) which inhibits irreversibly (by dialysis) yet gives *competitive*, or partially competitive curves in the Lineweaver-Burk method—i.e., appears to be displaced immediately by acetylcholine. . . . It may be inferred that while the carbamic esters described combine at the substrate-active center, mercuric ion does not; but that combination of substrate changes the affinity of the latter for the protein moiety of the enzyme. It is suggested that "competitive inhibition" need not imply competition with substrate for the same site of attachment to the enzyme.

Complexes of hemocyanin and of hemorythrin with small ions. I. M. KLOTZ * AND F. TIETZE.

Previous investigations (*Biol. Bull.*, **94**: 40, 1948) have demonstrated that the serum of *Limulus polyphemus* is capable of combining with small molecules other than those involved in its respiratory function. To establish that the binding is by the hemocyanin component of the serum, this protein has been isolated, and its ability to form complexes with several organic ions has been examined.

Quantitative estimates of the binding of methyl orange by isoelectric hemocyanin have been obtained from equilibrium dialysis experiments. At dye concentrations as high as 7×10^{-5} molar, an average of about 0.1 mole of anion is bound by each unit (of 37,000 molecular weight) of the hemocyanin. This is of the same order of magnitude as was found for an equivalent quantity of this protein in the serum.

Qualitative evidence of the binding of several other ions by hemocyanin has been obtained from spectrophotometric observations.Thus the spectrum of a mixture of Orange II and the protein differs from the sum of those for the pure components by a shift of 150 Å in the region of the dye's peak near 4850Å, as well as by an increased absorption in the 5800 Å region of the chromoprotein. With salicylate ion, in turn, which has no spectrum in the visible region, only an increased absorption in the 5800 Å band is observed. A small change in the absorption of solutions of the cationic dye, methylene blue, in the presence of hemocyanin has been found also, but its interpretation is doubtful, since the dye is aggregated, even at these low concentrations, and small shifts on addition of protein might be due merely to changes in the dielectric properties of the medium.

Hemerythrin, isolated from a sample of serum of *Phascolosoma gouldi* kindly given to us by Dr. Ernest Baldwin, shows even more pronounced evidence of complex formation with small ions. Thus in dialysis experiments with the anionic dye, Orange II, an average of 2.5 moles of ion is bound, by each unit of 100,000 molecular weight, at a free dye concentration of slightly less than 1×10^{-4} molar. (The unit of 100,000 molecular weight was chosen arbitrarily, for purposes of comparison, since adequate data on this protein are not available.) Similarly, in spectrophotometric studies, the absorption peak at 5000 Å is shifted over 500 Å to shorter wave-lengths, and the optical density is doubled, on the addition of thiocyanate to the protein. Similar effects

* Lalor Fellow.

are obtained with other known iron-complexing agents such as cyanide or hydroxylamine hydrochloride.

Thus it seems probable that many of the respiratory pigments can act as ion-transport agents, in addition to their primary function as oxygen carriers.

Further studies on the mechanism of alloxan action; the reaction of alloxan with sulfhydryl groups; the glutathione content of islet tissue. ARNOLD LAZAROW.*

It has been suggested that alloxan produces diabetes because of inactivation of essential sulfhydryl enzymes and that the selectivity of alloxan for the beta cells may be due to a low glutathione content (Lazarow, A., *Proc. Soc. Exp. Biol. and Med.*, **61**: 441, 1946).

Alloxan has been shown to react with glutathione and protein at pH 7.4 to give a new compound with an absorption spectra maximum at 305 mμ [Lazarow et al., *Science* (in press)]. When p-Cl-Hg benzoate is added to a glutathione solution prior to the addition of alloxan, no "305" is formed. Since p-Cl-Hg benzoate is a specific sulfhydryl reagent, the failure of formation of "305" indicates that alloxan is reacting with the sulfhydryl group of glutathione. These in vitro studies suggest the possibility of protecting animals against alloxan diabetes by injecting p-Cl-Hg benzoate prior to a diabetogenic dose of alloxan. Following the disappearance of the injected alloxan (spontaneous decomposition occurs at pH 7.4 with a half-life of 1 minute at 37° C.), it may be possible to reactivate the sulfhydryl groups by the addition of BAL, which removes the mercury compound.

Determinations have been carried out on the glutathione content of the islet tissue of fish. The ferricyanide method of Mason was adapted for micro-analysis in final volumes of 0.5 cc. The principal islet of the goosefish (*Lophius piscatorius*) contains 27–58 mgs. glutathione/100 gms. tissue (average glutathione of 9 fish was 50 mgs./100 gms.). This value is lower than the glutathione content of liver or kidney, and greater than that of muscle. Although the islet glutathione values represent the average glutathione content of the alpha, beta, and gamma cells, it may be possible to determine the glutathione content of the beta cells by indirect means [using the glutathione content of alloxan diabetic fish (alpha and gamma cells) and the percentage composition of cell types].

The insulin content of the islet tissue of alloxan diabetic fish. ARNOLD LAZAROW * AND JACK BERMAN.

Diabetes was produced in the toadfish by injecting alloxan subcutaneously in doses of 600 mgs./kg. body weight. Serial blood sugar determinations were carried out by the Folin-Malmros microblood sugar method. After the removal of the islet tissue, it was homogenized in ice-cold saline and serials dilutions were prepared. Doses, ranging from .05 to 1.0 mgs. of islet tissue, were injected into mice which had been starved for 24 hours (three animals were used for each dilution). Blood samples were drawn from the tail vein at 0 and 30 minutes after injection.

Using the islet tissue of normal fish, the percentage drop in blood sugar was found to be proporportional to the log of the injected islet dose. When liver homogenates or saline were injected into mice, under similar conditions, they resulted in an elevation of blood sugar.

The islet tissue of alloxan diabetic fish (sugars greater than 400 mgs./100 cc.), assayed 48 hours after the injection of alloxan, showed considerable quantities of a blood sugar lowering factor (insulin). (The injection of 0.1 mg. or less of islet tissue per mouse produced a 25–40 per cent drop in blood sugar.)

Although the limited number of analyses carried out so far do not permit accurate comparison between the insulin content of the islets of normal and diabetic fish, they do indicate that both may be of the same order of magnitude.

The presence of sizable quantities of insulin in the islet tissue of alloxan diabetic fish raises the question as to whether there may be a dissociation between insulin storage and insulin secretion, or whether factors other than insulin deficiency may play a role in the etiology of the hyperglycemia which persists after alloxan injection.

* Lalor Fellow.

A New Concept of the Action of Dicumarol. JOSEPH LEIN.[1] *

All previous work with Dicumarol has tended to confirm the view that the drug acts by decreasing the prothrombin content of the blood by preventing its synthesis in the liver. The evidence for this view has been obtained by determining clotting times of Dicumarol treated plasma using a protein thromboplastic agent. Such plasma shows a markedly increased clotting time.

In addition to protein thromboplastic agents there is also a lipid thromboplastic agent which has a considerably lower clotting activity than the protein with normal plasma. Experiments were carried out in which the clotting times of rabbits treated with Dicumarol were determined with the lipid and protein thromboplastic agents.

Three day treatment of rabbits with 10 mg. of Dicumarol per kg. per day caused a marked increase in the clotting time of rabbit plasma using the protein thromboplastic agent, while the clotting time obtained with the lipid agent increased but slightly. Thus, in one case the protein agent did not clot the plasma in over three hours while the lipid clotted it in five minutes. Comparison of the protein thromboplastic agent clotting times with control clotting times indicates further that the protein thromboplastic agent acts as an inhibitor of the clotting of Dicumarol plasma. Addition of highly purified prothrombin brings the relationships to those found in normal plasma.

Since reduction of prothrombin concentration of plasma by physical methods of dilution or absorption on aluminum hydroxide does not reverse the relative thromboplastic activities of the lipid and protein agents, it is believed that Dicumarol treatment does not prevent the synthesis of prothrombin by the liver but causes the synthesis of an altered prothrombin. This altered prothrombin is not converted to thrombin by the action of a protein thromboplastic agent but is converted by the action of a lipid agent.

Enzyme localization in the giant nerve fiber of the squid. B. LIBET.[2] *

Last summer it was found that the enzyme ATP-ase is localized almost exclusively in the sheath of the giant axon, with practically none in the axoplasm. This sheath ATP-ase showed a rate of activity even greater than that of squid muscle, and also certain other similarities to muscle ATP-ase. A further analysis of the properties of this nerve ATP-ase shows the following: (a) The rate of activity is increased by increasing concentration of either Ca or Mg ions, up to a maximum. (b) The maximum activity is greater with Mg and occurs at a lower concentration (about 0.003 M for Mg is almost maximal; about 0.03 M for Ca). (c) Mg stimulates at all K ion concentrations (up to 0.58 M), unlike the situation in muscle, where it inhibits at high K. (d) When Ca and Mg are added together there is antagonism, but in the reverse direction from that reported for muscle ATP-ase; i.e., Ca inhibits the Mg activation in nerve.

Neither sheath nor axoplasm splits any other phosphate esters tested at an appreciable rate. β-glycerophosphate is split at a very low rate even at pH 9.1 in the presence of Mg and glycine, so that "alkaline phosphatase" is very low. ATP-ase seems to be the dominant phosphatase present.,

An attempt was made to localize further the ATP-ase within the sheath itself by separating the axolemma from the rest of the sheath, but this has yielded inconclusive results thus far. Since the sheath of the giant nerve fiber contains a high percentage of connective tissue (C. T.), ATP-ase was determined in muscle-free C. T. samples taken from the mantle where the latter faces the dorsal side of the pen of the squid.[3] This muscle-free C. T. showed an ATP-ase activity of about 7 μg. P/mg. wet wt./30 min. at 27° C. (compared to a usual figure of about 15–20 for the axon sheath); it is activated by Ca^{++} and does not split β-glycerophosphate or hexose-di-phosphate appreciably. If one could assume that the C. T. of the axon sheath has an activity identical with that of this muscle-free C. T., considerable activity would still be left for the non-C. T. elements. An attempt at further localization will be made histochemically by

* Lalor Fellow.

[1] Department of Zoology, Syracuse University, Syracuse, New York.

[2] This work was also supported in part by the Navy Nervous System Research contract with the University of Chicago.

[3] I am indebted to Dr. Magnus Olson for identifying the muscle-free C. T. Only a trace of muscle, that in small blood vessel walls, was found.

Dr. G. Gomori. Obviously results obtained by others on the distribution of other enzymes between sheath and axoplasm in the giant nerve fiber must also be re-examined in the light of these findings.

The choline acetylase and choline esterase content of some invertebrate tissues. HAROLD PERSKY * AND MARCIA GOLD.

The hypothesis that acetylcholine plays the primary role in the generation of the nervous impulse requires that the enzymes synthesizing and decomposing acetylcholine (ACh) be present in significant amounts in all animals possessing a differentiated nervous system. In order to test this premise, the choline acetylase (ChAc) and choline esterase (ChE) content of representatives of the three phyla with the most primitive nervous systems were determined. *Tubularia crocea* (Coelenterate), *Euplanaria maculata* (Platyhelminthes) and *Neanthes virens* (Annelida) were the organisms studied. The entire organism was employed in the first two animals because of the difficulties associated with the dissection of the nervous system in these species. The first segment of *Neanthes* was used because it contains the "brain." The methods of assay for both enzymes were essentially those of Nachmansohn. All the results are expressed as milligrams acetylcholine formed or split per hour per hundred milligrams protein nitrogen (Q). The data is summarized in the following table.

Species	Q_{ChAc}[1]	Q_{ChE}[1]	Q_{ChE}/Q_{ChAc}	$\frac{\text{Protein nitrogen}}{\text{Wet weight}} \times 10^2$
Tubularia crocea	0.03(0.002)	180(1.3)	∞	0.75
Euplanaria maculata	23. (0.25)	2000(20.)	85	1.1
Neanthes virens	20. (0.3)	280(4.2)	14	1.5

[1] Figures in parentheses are the Q values expressed per 100 mg. wet weight. All data at 20° C.

It is apparent from the data that choline esterase is present in all three species in amounts equal or greater to that in rat brain (Q_{ChE} of 210). Since the ChE of these two species is the nerve-muscle type (Nachmansohn), the concentration of ChE in the nervous tissue of these two species may be from 10^3 to 10^4 times that of vertebrate brain. The problem of why such large amounts of ChE are present in such relatively poorly developed nervous systems requires greater study.

The choline acetylase determinations posed greater technical problems than the ChE assays. Acetylcholine synthesis has not yet been shown to be proportional to enzyme content in vertebrate brain or nerve or even highly purified choline acetylase. However, by employing low tissue concentrations, short incubation periods and low temperatures, first order responses between enzyme activity and enzyme content were obtained in the case of *Euplanaria* and *Neanthes*. No such proportionality was obtained in the case of *Tubularia*. In all cases, the absolute amount of acetylcholine synthesized was small. In spite of these shortcomings, the ChAc content of *Euplanaria* and *Neanthes* exceeded vertebrate brain. *Tubularia* showed little ChAc content despite its high ChE content.

The ratio of ChE to ChAc showed no constancy for the three species; rather the ratio progressively decreased as the nervous system became more highly organized. The problem as to whether this change is related to the functional state of the nervous system is worthy of further investigation.

Report of Investigations, Summer 1948. AVRAM GOLDSTEIN * AND DORA B. GOLDSTEIN.

I. *Non-nerve cholinesterases.* No reasonable function can as yet be attributed to the cholinesterases found in the plasma and liver of higher animals. This project sought to isolate and

* Lalor Fellow.

study the properties of a cholinesterase in a system where it would be known to have a definite metabolic function—e.g. in microorganisms. Fermenting cabbage, cucumber and algae (such materials had been shown to contain bacteria which *produce* acetylcholine—M. Stephenson, *J. Gen. Microbiol.*, 2, No. 1, 1948) were used as sources for the isolation by enrichment culture, of organisms which might utilize acetylcholine. Forty pure cultures were obtained (representing at least twelve different species) which grew in several passages through acetylcholine in mineral medium. These include Gram-negative bacilli, yeasts and molds. To date, growth and respiration studies reveal that some (but not all) metabolize added acetylcholine, grow on choline (but not on ammonia plus acetate) and hydrolyze acetylcholine anaerobically. One organism has yielded an acetylcholine-splitting enzyme in acetone powder. This esterase has not yet been characterized but it is insensitive to prostigmine. The work will be continued during the coming year.

II. *Modified Warburg technique.* Theoretical work was completed and experimentally confirmed for a variable-volume use of the standard Warburg apparatus. The method eliminates the necessity of repeatedly levelling the manometer fluid, increases the capacity for measuring gas uptake or liberation, and makes possible automatic recording of the progress of reactions.

III. *Further kinetic studies on the mechanism of inhibition of human plasma cholinesterase.* See Seminar, presented August 17, 1948.

The incorporation of P^{32} into the nucleoproteins and phosphoproteins of developing Arbacia embryos. C. A. Villee,* M. Lowens, M. Gordon, E. Leonard, and A. Rich.

The quantitative conversion of ribonucleic acid (RNA) to desoxyribonucleic acid (DNA) in the developing sea urchin embryo was postulated by Brachet (1933) on the basis of an increase in DNA and decrease in RNA during the first 40 hours of development. Schmidt (1948) reinvestigated this with more precise analytical methods and found an increase in DNA but no decrease in RNA.

Sea urchin embryos obtained at stages from 3 to 72 hours of development and analyzed by the method of Schmidt and Thannhauser (1945) showed an increase in DNA with time whereas the RNA remained constant or increased slightly. The major source of the DNA formed cannot be the RNA of the unfertilized egg because when fertilized eggs are allowed to develop in sea water containing P^{32}, a great amount of radio-phosphorus is incorporated into DNA, which would not be the case if it were derived from the P^{32}-free RNA of the unfertilized egg. A comparison of the specific activities of the two fractions showed that for DNA to be formed from an RNA intermediate, less than 5 per cent of the RNA must be active in forming DNA and this small fraction must be synthesized and broken down completely more than four times in 24 hours.

A number of chemicals which have been found to have effects on phosphate metabolism were tested for their effects on phosphate uptake and on nucleoprotein synthesis by comparing the specific activity of the fraction of the treated with that of the control animals grown at the same time and under the same conditions. 5×10^{-3} M dinitrophenol and 10^{-2} M malononitrile inhibited cleavage so that after 12 hours only 1 or 2 cell stages were present, whereas the control embryos were free swimmers. Both 10^{-2} and 10^{-4} M malononitrile inhibited respiration to about 25 per cent of the control value. Uranyl nitrate and tris (β chloroethyl) amine hydrochloride inhibited cleavage but not, at the concentrations used, as much as dinitrophenol.

The table gives the effects of these substances on the incorporation of P^{32} into the several fractions after 12 hours of incubation. Dinitrophenol appears to inhibit the uptake of phosphorus by the cell (the acid-soluble phosphorus fraction is low) more than the synthesis of DNA and RNA. Low temperature, which inhibited cleavage completely, inhibited the uptake of phosphorus into the acid-soluble fraction about as much as dinitrophenol, but inhibited the synthesis of nucleoproteins much more. Malononitrile inhibited the uptake of phosphorus into the acid-soluble fraction to a lesser extent but inhibited DNA and RNA synthesis markedly. RNA synthesis appears to be more sensitive to malononitrile than DNA synthesis, which suggests that DNA is not formed from an RNA intermediate. Uranyl nitrate reduces the uptake of phosphorus and inhibits both DNA and RNA synthesis. The nitrogen mustard, tris (β chloroethyl)

* Lalor Fellow.

amine inhibited both DNA and RNA synthesis, DNA more than RNA, without reducing the uptake of phosphorus by the cell to any marked extent.

TABLE 1

Inhibition of the incorporation of P^{32} into the phosphate fractions of 12 hour Arbacia embryos

The figures in the table represent the values of the ratio:

$$\frac{\text{Specific activity (counts per minute per mg. P) of treated embryos}}{\text{Specific activity (counts per minute per mg. P) of control embryos}}$$

	Malononitrile		Dinitrophenol	Uranyl nitrate	Nitrogen mustard	Cold
	10^{-4} M	10^{-3} M	5×10^{-5} M	10^{-4} M	10^{-4} M	0° C.
Acid soluble P	.69	.49	.18	.49	.83	.16
Total acid insoluble P	.68	.10	.32	.40	.56	.06
DNA phosphorus	.55	.04	.10	.27	.23	.03
RNA phosphorus	.21	.03	.24	.40	.44	.03
Phosphoprotein P	.24	29	.16	.55	2.57	.23

Studies on nucleoproteins from marine invertebrates.[1] C. A. VILLEE,* E. LEONARD, AND A. RICH.

The studies on marine invertebrate nucleoproteins begun last summer were continued. Nucleoproteins were extracted from squid testis, spermatophore sac, and sperm receptacle using 2M NaCl. Purified solutions of these have a high relative viscosity and show strong flow birefringence. Measurements of the flow birefringence were made in the Mehl and Edsall apparatus. Changing the pH of these solutions to pH 3.6 or 8.5 did not decrease the viscosity or flow birefringence. Heating to 100° C. for 40 minutes did not decrease viscosity or birefringence but heating for 90 minutes at that temperature did destroy birefringence and decreased the viscosity markedly. There was no change in these properties on the addition of guanidine hydrochloride up to 20 mM/ml. solution, which suggests that these properties do not depend on the presence of free nucleic acid. Observations from the viscosity and flow birefringence data indicate that the nucleoproteins are present as markedly asymmetric particles. Using as a model an ellipsoid of revolution, calculations show a rotary diffusion constant of 1.5 and a particle length of about 10,000 Angstroms. It is possible that the nucleoprotein molecules are associated into larger particles under these conditions.

Analyses by the Schmidt and Thannhauser method for ribonucleic acid, desoxyribonucleic acid, and phosphoprotein were made on squid testis, spermatophore sac and on squid optic ganglion. The effect of continued stimulation on the nucleoprotein content of squid optic ganglia was investigated. Nitrogen: phosphorus ratios were determined on nucleoproteins and nucleic acids prepared from squid, starfish, and sea urchin tissues.

* Lalor Fellow.

[1] Aided by a grant from the Ella Sachs Plotz Foundation.

PAPERS PRESENTED AT THE MEETING OF THE SOCIETY OF GENERAL PHYSIOLOGISTS

The wave of negativity produced by acetylcholine conducted over an oil-saline interface. T. C. BARNES AND R. BEUTNER.[1]

The wave of negativity generated by acetylcholine at a phase-boundary has been found to travel from the point of application of the drug to the recording non-polarizable electrodes placed a meter or more distant. An oil-saline interface is essential for the transmission of this wave since it will not cross a saline bridge. The aqueous medium was 0.9 per cent sodium chloride. In some cases sodium lauryl sulfonate or sodium benzoate was used to increase the sensitivity of the oil surface to the alkaloid. The elongated interface consisted of a glass tube containing the two layers with openings at one end for the introduction of acetylcholine and at the other for the electrodes leading to the electroencephalograph. The addition of 0.1 cc. of saline containing 0.1 mg. acetylcholine at the "entrance" produced a negative wave of 3 millivolts lasting 0.15 sec. recorded at the "exit" 80 or more cm. away. The glass tube can be replaced by two contiguous wicks, one containing oil and the other saline. A single wick of oil or a single wick of saline will not transmit the acetylcholine wave. The mechanism of transmission is being investigated. Controls of distilled water or saline applied to this artificial nerve do not produce the wave. An electrogenic organic base (acetylcholine mecholyl or thiamine) is essential to establish the initial phase boundary potential. It will be noted that no "membrane" potential is necessary for initiation or transmission of the wave of negativity. The acetylcholine potential was originally set up as a permanent "base-line shift" potential (Beutner and Barnes, *Science*, **104**: 569, 1946), later developed into a wave (Barnes, *Federation Proceedings* **6**: 73, 1947), and the experiments reported here describe the transmission of the wave. It is possible that nerve impulses and brain-waves are produced by a similar mechanism.

Apyrase activity of invertebrate marine muscle. ARTHUR COHEN [2] (*introduced by H. B. Steinbach*) (*by invitation*). READ BY TITLE.

A comparative study was made of the rates of hydrolysis of adenosine-triphosphate by unpurified homogenates of various marine invertebrate muscles. The results of these tests are shown in the table below in which the values are expressd as micrograms of phosphorus liberated from ATP by 1 mg. of fresh muscle in 5 and 15 minute incubation periods. No significant relationship between smooth and striated muscles, the nitrogen content of the muscles, or their activity, could be derived from the wide range of enzyme activity. In each assay of enzyme activity approximately 0.5 g. of freshly excised muscle was homogenized in 5 cc. ice cold distilled water and 0.1 cc. aliquot taken for analysis. The calcium salt of ATP was used with final concentration of 0.0015 M. The labile 7′ P of the ATP was at least double the concentration of the P split by the enzyme at all times during the experiment. The pH of the medium was equal to 7.4 employing a veronal acetate buffer. The phosphate determinations were made by the Fiske-Subbarow method. For total nitrogen determinations the modified micro-Kjeldahl method of Ma and Zuazaga was followed. Incubation temperature of the enzyme mixture was 22° C.

Muscle	μ P/mg./5 min.	μ P/mg./15 min.	μ Total N/mg.
Thyone (retractor)	0.1	0.34	18.0
Phascolosoma (retractor)	0.5	0.90	21.2
Limulus (tail)	0.62	1.50	28.2
Pagurus (abdominal)	2.8	4.20	29.6
Pecten (adductor)	2.8	4.73	22.6
Mya arenaria (mantle)	3.6	5.88	29.5
Loligo Pealii (fin)	4.7	9.00	25.3

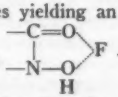
[1] Department of Pharmacology, Hahnemann Medical College.
[2] University of Minnesota.

*On the nature of iron binding by siderophilin, conalbumin, hydroxylamine, aspergillic acid, and related hydroxamic acids.** SILVIO FIALA AND DEAN BURK.[1]

Aspergillic acid, hydroxyaspergillic acid, N-hydroxy, 4-methylpyridene-2, N-hydroxy, 5-bromopyridene-2, N-hydroxy, 4,6-dimethylpyrimidene-2, and hydroxylamine have been found to bind ferric iron under appropriate conditions to yield complexes with the same absorption spectrum maximum (460–465 mμ) and order of extinction at this maximum (0.025–0.05 per cm. at microgram complex-Fe per ml.) as do the specific salmon-pink complexes of iron with conalbumin of egg white (Schade and Caroline, *Science*, **100**: 14–15, 1944) and of iron with siderophilin, the iron-binding β_1-pseudoglobulin of human plasma (Schade and Caroline, *Science*, **104**: 340–341, 1946; Helmberg and Laurell, *Acta Chem. Scand.*, 1: 940–950, 1947; Surgenor, Strong, and Koechlin, *J. Clin. Invest.*, in press; Schade, Reinhart, and Levy, *Arch. Biochem.*, in press).

Carbon dioxide (bicarbonate) is required to obtain the salmon-pink complex with iron and hydroxylamine, as Schade, Reinhart, and Levy first found for the iron complexes of conalbumin and siderophilin, the stoichiometric ratio of CO_2 to Fe in the latter being 1 : 1. With aspergillic acid and the related cyclic compounds listed, however, no carbon dioxide is required, consistent with the existence already of a carbonyl group adjacent to a hydroxylated nitrogen atom in the cyclic hydroxamic acid grouping that Dutcher (*J. Biol. Chem.*, **171**: 341–353, 1947) has indicated to be responsible for iron binding. The ratio of C=O to Fe in the salmon-pink aspergillic acid iron compound is 1 : 1.

Hydroxyl ion is also required to form the salmon-pink complexes of siderophilin and conalbumin, but it is not yet required by hydroxylamine and the cyclic hydroxamic acids listed, which already contain OH groups attached to nitrogen. The shape of the titration curve of the salmon-pink siderophilin complex, in the presence of buffer such as phosphate or citrate, indicates a ratio of OH^- to Fe of 1 : 1.

The grouping in the siderophilin, conalbumin and other complexes yielding an absorption spectrum maximum in the region 460 mμ might thus be represented as  . The specificity of the two proteins as compared with other proteins tends further to rule out primary involvement of the more common sulfhydryl and amino groups, which might otherwise be suspected of iron combination. Physiological implications will be discussed.

The cytochrome system in relation to diapause and development in the Cecropia silkworm.† CARROLL M. WILLIAMS AND RICHARD C. SANBORN.[2]

As soon as the pupa is formed, the metamorphosis of the Cecropia silkworm is interrupted by a prolonged period of diapause, which, at room temperature, continues for not less than five months. During this period the animal persists in a state of developmental standstill. The termination of dormancy is under overall control, being evoked by a combination of two internal factors. One of these arises from the pupal brain, the other from the prothoracic glands.

As a working hypothesis we may assume the existence of a biochemical defect in the tissues of the diapausing pupa that prevents their metabolism from contributing to morphogenesis. From this point of view, the brain and prothoracic glands should preside over some synthetic reaction whereby the tissues repair this defect.

Certain properties of the metabolism during dormancy and during development indicate that the biochemical defect of diapause may involve iron-catalyzed oxidations. Thus, during diapause, the oxygen consumption is essentially insensitive to cyanide. But when the brain and prothoracic glands function to terminate diapause, the oxygen consumption increases and a progressively

* The siderophilin (plasma fraction IV-7) employed in this study was kindly supplied by Professor E. J. Cohn and Dr. Douglas Surgenor, Department of Physical Chemistry, Harvard Medical School, the conalbumin by Dr. H. L. Fevold, Western Regional Research Laboratory, and the aspergillic acid, hydroxyaspergillic acid, and related hydroxamic acids by Drs. J. D. Dutcher, O. Wintersteiner, and W. A. Lott of the Squibb Institute for Medical Research.

[1] National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

† This study was aided by the Lalor Foundation.

[2] The Biological Laboratories, Harvard University.

larger fraction becomes cyanide sensitive. Consequently, development seems to involve a progressively larger utilization of the cytochrome system.

This possibility has been tested by spectrophotometric assay of cytochrome C and manometric assay of cytochrome oxidase. A striking correlation was found between the titer of these enzymes and the progress of adult development. Thus, cytochrome C is virtually absent from the diapausing pupa. During the period when development is dependent on the prothoracic glands, the concentration of cytochrome C increases from less than 1 to more than 50 gamma per gram live weight. Similarly, during the period of the brain's secretory activity, the titer of cytochrome oxidase increases from approximately 40 to nearly 700 units.

Two correlations seem to emerge; namely, that between the synthesis of cytochrome oxidase and the function of the brain, and that between the synthesis of cytochrome C and the function of the prothoracic glands. As a result of the combined functions of both the brain and prothoracic glands, the tissues of the dormant pupa, for the first time, come into possession of a complete cytochrome system.

The relation of heparin to protoplasmic clotting. L. V. HEILBRUNN AND W. L. WILSON.

An essential part of the present-day theory concerning the colloidal behavior of protoplasm is the fact that the gelation of protoplasm is in many ways similar to the clotting of blood. The changes which protoplasm undergoes during its normal activities and as a result of drug action are frequently to be explained in terms of a clotting reaction similar to that which occurs in blood. Additional evidence in support of this point of view is the fact that at least in some cells heparin can prevent protoplasmic gelation. In the egg of the worm Chaetopterus, there is a gelation which precedes the appearance of the mitotic spindle. If eggs are exposed to dilute solutions of heparin and then fertilized, the mitotic gelation is inhibited and for the most part the eggs do not divide. In order to show this, it is necessary to inseminate the eggs with high concentrations of sperm, for the presence of heparin tends to inhibit fertilization by weaker sperm concentrations. The effect of heparin on cell division is reversible. The fact that heparin can actually prevent mitosis is perhaps of some significance in the interpretation of the effect of radiation in the treatment of cancer, for following irradiation of animals, there is a marked increase in the amount of heparin in the blood stream. Moreover, heparin may be an important factor in the control of protoplasmic clotting in various other types of cell activity.

*Enzyme activity and radiation sensitivity of enzyme-substrate films.** DANIEL MAZIA AND GERTRUDE BLUMENTHAL.[1]

In a previous investigation (Mazia, Hayashi, and Yudowitch, Cold Spring Harbor Symp., 1947) the enzyme activity of mixed surface films of pepsin and albumin has been described. In all such experiments it has been difficult to exclude the possibility that the measured activity was not that of surface-spread enzyme molecules but of unspread molecules trapped in or adsorbed on the film. It has been found (Mazia and Blumenthal, *P. N. A. S.*, 34: 328, 1948) that these enzyme-substrate films are extraordinarily sensitive to radiation, and a study of the relation between activity, surface pressure, and radiation sensitivity throws light not only on the mechanism of radiation sensitivity but also on the question of the activity of surface-spread enzyme.

Methods are described in the publications cited. Surface pressure was measured by the vertical-pull balance. The activity of the films depends on the pressure against which they are spread. Films spread to zero pressure become inactive. Activity increases with initial pressure up to about 30 dynes/cm. There is some evidence of reversibility. The radiation-sensitivity is a function of the surface pressure. At a given dose, there is a discontinuous relationship between inactivation and pressure. Dose-inactivation curves at various pressures yield a family of sigmoid curves which show that the effect of pressure is primarily on the "threshold" of the dose-effect relationship. A plot of pressure against dose for 50 per cent inactivation yields a straight line. The 50 per cent dose at 7 dynes/cm. is about 100 r; at 35 dynes/cm. it is 500 r. The

* Work supported by National Research Committee on Growth acting for the American Cancer Society.

[1] Department of Zoology, University of Missouri.

pressure effect on sensitivity is reversible. Sensitivity depends on the pressure at the time of radiation and is indpendent of previous pressure changes provided the pressure has not been permitted to fall to zero. Since not only the activity but also radiation-inactivation is a function of surface pressure it is concluded (1) that the spreading of proteins at an air-water interface ("surface denaturation") is not necessarily an all-or-none phenomenon, but may be limited by the pressure against which the protein spread and (2) that if the unfolding of the molecule is not permitted to go to completion (i.e. to zero pressure) the enzyme activity of the protein may be preserved.

A photosynthetic intermediate. A. H. Brown, E. W. Fager and H. Gaffron.[1]

An intermediate of photosynthesis is by definition a substance which is transformed in one or more photochemical steps into the final product. This final product of photosynthesis must have the reduction level of a carbohydrate and be insensitive to further irradiation. The discovery of the dark fixation reactions in which free carbon dioxide becomes a carboxyl group in an organic molecule without the aid of light made it very probable that the first photosensitive intermediate in the course of photosynthesis is formed by a typical dark fixation and thus may be related to compounds which normally serve as intermediates of respiration. Consequently many biochemists expected photosynthesis to be revealed as a process in which intermediates known from the breakdown of carbohydrates are built up in steps which literally reverse the course of respiration.

Benson and Calvin have reported that in studying photosynthesis with radioactive carbon as a tracer they have obtained labeled dicarboxylic acids, amino acids and very considerable quantities of glyceric acid and of glyceraldehyde. According to these authors, these substances have been formed by the direct photosynthetic reduction of carbon dioxide, that is, neither by the breakdown of a previously synthesized carbohydrate nor by way of an ordinary dark fixation. The intimate relationship of respiration and photosynthesis as well as the pathway of the latter process thus seems to have been conveniently established.

In the course of our investigations with carbon 14 we could confirm most of the early observations of Ruben, Kamen and Hassid, but could not find any indication that the substances mentioned by Calvin et al. are present as intermediates of photosynthesis. Even in the case where these authors adopted the technique of short exposures and found glyceric acid and glyceraldehyde as main products, the discrepancy persists. Under the same conditions we obtain 90 per cent of the assimilated carbon in one chemical fraction of the plant. This fraction contains the carbon in a photosensitive substance (or group of nearly related substances) which is not identical with any one of the substances usually shown in the schemes representing the metabolism of carbohydrates. Labeled glyceraldehyde is absent, and glyceric acid is present, if at all, only in small amounts, which probably originate from secondary processes. The intermediate is obtained as a thermally and chemically very stable though impure syrup or its hygroscopic sodium or barium salts. It is easily adsorbed on all kinds of precipitates when these are produced in the solution. Its most interesting characteristic is its content of aromatic nitrogen. The ultraviolet spectrum is very similar to that of uracil, though this particular substance is not present. It seems that the radioactive carbon assimilated during the first moments of photosynthesis and a pyrimidine compound are closely associated. The chemical properties which set our photosynthetic substance apart from the better known simple metabolites are paralleled by its behavior in the living cell. Instead of being rapidly transformed in the dark like any respiratory intermediate it is stable against attack by respiration. It also does not readily exchange its labeled carbon with free carbon dioxide, as it should if it were a primary dark fixation product. But the labeled carbon begins immediately to appear in other chemical fractions when the plant is exposed to light. These results do not support the idea that the photochemical reduction of carbon dioxide proceeds by a simple reversion of each step in the breakdown of carbohydrates.

Synthesis reactions with acetic acid in isolated bone marrow. Richard Abrams, J. M. Goldinger, and E. S. G. Barron.

The metabolic activity of bone marrow has been the subject of much interest because of its role in blood cell production. It has been shown by Thorell (1947) that ribonucleic acid is

[1] University of Chicago, Chicago, Illinois.

associated with protein formation in the proliferating marrow; by Goldinger, Lipton, and Barron (1947) that marrow can oxidatively utilize acetate, and by Buchanan, Sonne, and Delluva (1947) that acetate carboxyl can be a purine precoursor (uric acid in pigeons). Accordingly, we have incubated rabbit bone marrow slices and homogenates with acetate containing C^{14} in the carboxyl group in an attempt to find an *in vitro* system for studying nucleic acid synthesis, as well as to survey the types of synthetic reactions in which acetate is involved. A small, but definite, incorporation of C^{14} was found in both ribonucleic and desoxyribonucleic acids. In addition there was observed a relatively large turnover in the phospholipid and protein fractions.

In a typical experiment, rabbit marrow slices were incubated for 5 hours at 37° in Ringer-bicarbonate containing $CH_3C^{14}OONa$. Taking 100 as the specific activity of the substrate, the relative specific activities of the subsequently isolated fractions were: ribonucleic acid (RNA), 0.035; desoxyribonucleic acid (DNA), 0.014; CO_2 liberated from hydrolyzed protein by ninhydrin, 0.61; lecithin, 0.90; and fatty acids from saponified fat, 0.10. A control experiment with $C^{14}O_2$ indicated that the startlingly high rate of protein formation from a fatty acid (as well as phospholipid synthesis) did not involve CO_2 as an intermediate.

The rate of incorporation of C^{14} was measured aerobically with slices and both aerobically and anaerobically with homogenates. With slices, there was a linear increase of specific activity with time in the DNA and amino acid carboxyl fractions, while the RNA activity, though reaching a level 3 to 4 times that of DNA, did so at a continuously diminishing rate. Homogenization caused a marked reduction in the rates of uptake of C^{14} by RNA and by protein. That respiration is necessary to furnish the energy for these synthetic processes is indicated by the fact that anaerobically C^{14} appeared in neither the nucleic acids nor the proteins.

Thermodynamic theory of the contraction of actomyosin. A. SZENT-GYÖRGYI.[1]

It has been shown in the speaker's laboratory that the contractile matter of muscle is built of a complex protein, actomyosin, composed of myosin and actin (F. B. Straub). Actomyosin contracts in a proper ionic milieu under influence of ATP. Evidence was obtained showing that the contractile matter is built of small functional units each of which consists of a certain amount of myosin, actin and one molecule of ATP. These units will be called "autones." Experiments indicated that these autones have but two stable states, the fully relaxed and fully contracted state; that contraction is an all-or-none process; and that it is an equilibrium-process, dependent on temperature. The equilibrium-constant was measured at different temperatures and free-energy changes calculated. It has been found that the ΔF, i.e. the free-energy spent by the single autone in contraction, rises with increasing temperature. In the rabbit it is 0 at about 0° C. and reaches 11,000 cal. at 53° C. In the frog-muscle, extracted with water, these values are reached a few degrees lower.

The first question is whether this ΔF curve is correct. The theory involves that the system uses its own F in contraction and extraneous energy is needed for relaxation. The extraneous source of energy is the high-energy phosphate of ATP which has 11,000 cals. of F. It follows that the muscle has to go over permanently into the contracted state at the temperature where the F-expenditure reaches or exceeds 11,000 cal., which is 53° in the rabbit and 47° in the frog. This was actually found to be the case.

In order to obtain further information about the correctness of the theory the total amount of work was measured in isometric and isotonic contraction. It was found that the curve of total work, if calculated for 35,000 gm. of myosin, agrees very closely with the ΔF curve. This is the case in isotonic contraction and in isometric contraction up to the point where the work corresponds to 5500 cal., ½ of 11,000. From this point on the tension developed remains constant and does not rise with increasing temperature.

The thermodynamic reversibility of contraction could clearly be demonstrated.

Having thus obtained evidence for the basic correctness of the theory an attempt was made to extend it. If we suppose that actomyosin, in absence of ATP, does not contract because the F of the contracted state is equal with that of the relaxed state, $F_c = F_r$, then contraction takes place with ATP because this latter makes $F_r < F_c$ which pushes the system towards a new equilibrium with a greater number of contracted units. Such assumptions can be tested by deriving their consequences and checking them in the experiment.

[1] Marine Biological Laboratory, Woods Hole, Mass., and the National Institute of Health, Bethesda, Md.

$F_e = F_r$ involves an equilibrium constant of 1 which means that at the temperature in question there is one contracted unit for every relaxed one, even in rest. The contracted units, being folded, can be expected to be highly elastic, the relaxed ones to be inelastic. The relative length of units in the folded and relaxed state is 1:7. The limit of elastic extensibility is reached thus when the folded units are fully extended which is reached at 175 per cent of the equilibrium length. The experiment showed that this is actually the case.

The muscle is highly elastic only while it contains its normal amount of ATP. If this is decomposed the muscle becomes inelastic. That the change is actually due to ATP can be shown by adding ATP to the inelastic washed muscle fibers which, under influence of ATP, become highly elastic again. The elasticity of fresh muscle proves that ATP is present in an active form, linked to actomyosin.

If the fully contracted autone is stretched it offers in the beginning little resistance. Then the resistance becomes proportional to stretching (Hooke region) and in the end tension exceeds stretching. If the uncontracted units contract in an isometric contraction the contracted units are stretched. If the muscle would be at equilibrium-length it could in the beginning develop no tension. Rest-length means a stretch of these units which brings the muscle into the Hooke region. The maximum of tension is reached when all units are half-extended and tension is one-half the maximum theoretical value as has actually been found in the experiment. No higher tension could be developed by the muscle without its permanent damage. If this one-half tension is exceeded, slipping begins, and if the full tension is reached, the muscle tears.

The size of the autones can be approached in different ways. All observations indicate that it contains 18,000 gm. myosin, one molecule of ATP and 6000 gm. of actin. The actin and myosin in this unit are linked by one SH bridge. It is not impossible that these single autones also form higher units, containing one molecule actin of MW 70,000 gm. and thrice that amount of myosin.

Usnic acid, an antibiotic, and sperm metabolism. LEONARD NELSON, *by invitation.* READ BY TITLE.

The effect of Usnic acid on the respiration of Arbacia sperm was determined manometrically. This antibiotic had previously been shown to inhibit cleavage and P^{32} uptake in fertilized eggs of the same species, but had little effect upon their oxygen consumption (Marshak and Harting, J. C. C. P., 1948). However, the same concentration of Usnic acid pronouncedly affected the sperm respiration. This effect was found to be dependent upon the density of the sperm suspension. The sperm concentration was measured spectrophotometrically—the optical density being directly proportional to the logarithm of the number of sperm.

A concentration of 1 mg. of Usnic acid per 100 cc. of 0.01 per cent gelatin-sea water was employed throughout, while the sperm concentration was varied from 100,000 to 1,000,000 sperm per cubic millimeter. At 25° C., the respiration was completely inhibited in the low concentrations and in the intermediate and high concentrations there was a marked increase in oxygen uptake.

Sperm count / Sperm No./c.mm.	Per cent control		
	30 min.	90 min.	180 min.
100,000	0	0	0
180,000	100	0	0
250,000	470	375	280
410,000	565	590	660
1,120,000	470	500	500

Preliminary observations seem to show that these effects are not attributable to change in motility inasmuch as after a given interval, treated and control sperm had moved an equal distance in capillary tubes.

These experiments seem to indicate that some energetic process apparently independent of sperm motility and distinct from oxidative cycles in the egg is being affected. Similar results were obtained with Asterias sperm.

Temperature coefficients of Apyrase systems from muscles of different animals. H. BURR STEINBACH. READ BY TITLE.

The Apyrase activities of unfractionated homogenates of muscle were determined at various temperatures within the range zero to thirty degrees C. Reaction mixtures of 2 ml. volume contained 0.05 M barbital buffer, pH 7.4, ATP (as the Ca salt), 2×10^{-4} M as well as the enzyme preparation. Checks with some of the tissues with glycerophosphate as substrate showed negligible activity toward this simple ester. Ten minutes reaction time was used, the reaction being stopped by adding trichloracetic acid to a final concentration of 5 per cent.

Logarithms of rates at different temperatures plotted against either degrees C. (for Q_{10} calculations) or the reciprocal of the absolute temperature approximated straight lines with a few instances which might be interpreted as breaks at critical temperatures near 15° C. Average results for Q_{10} were as follows: for fish (*Clinostomus elongatus* and *Lepomis macrochiris*) 1.4 to 1.6, frog (*Rana pipiens*) 1.7, mouse 1.8 to 2.0, bird (*Passer domesticus*) 2.1 to 2.2, and turtle (*Chrysemys marginata*) 2.1 to 2.2. Further study would be necessary before concluding that these are real genetic differences between the animal groups. Preliminary experiments indicated that the Q_{10} values bore no particular relationship to the environmental temperatures of the animals since fish kept at 22° C. for one month gave preparations having the same Q_{10} as similar fish kept at 0 to 5° C. for a similar period. Likewise, tropical fish (*Xiphophorus hellerii* Heckel) had temperature coefficients nearly like those of cold water minnows.

A few observations of apyrase activity of brain homogenates of fish, mouse and frog showed uniformly low Q_{10} values of about 1.4.

Studies of the kinetics of potassium exchange between cells and plasma of canine blood in vitro using K^{42}. C. W. SHEPPARD AND W. R. MARTIN.[1]

Freshly drawn heparinized canine whole blood is equilibrated *in vitro* (paraffin lined vessels) at 38.1° C. with a normal pulmonary atmosphere saturated with water vapor. It is then tagged by mixing with a small amount of plasma containing dextrose and radioactive KCl. The potassium content of the resulting plasma is thus raised by not more than 3 per cent of the normal and the blood sugar to about 400 mg. per cent. By this procedure cells are maintained in a healthy state for periods up to 10 hours as shown by minimal hemolysis, nearly constant hematocrit and minimal potassium leakage.

In whole blood the activity of the plasma decreases initially at a rapid rate which is correlated with the appearance of activity in the cells (red cells, white cells, and platelets). The initial decrease follows an exponential curve to an elevated base line, half of this change being complete in about 50 minutes. However, if the white cells and platelets be previously removed by repeated differential centrifugation the behavior is different. The plasma activity declines very slowly, at the rate of about 1 per cent per hour.

The initial rapid decrease in plasma activity for whole blood is attributed to a rapid exchange of potassium in the fraction containing the white cells and platelets. A typical value for the amount of this easily exchangeable potassium is about 30 per cent of the total potassium in the plasma. It is evident that studies of potassium exchange of erythrocytes must give equivocal results unless the white cells and platelets are removed in advance or otherwise taken into account.

[1] Oak Ridge National Laboratory, Oak Ridge, Tennessee.